

Shaw 2 Final Report- Residential Carpets

Customer Name	Shaw Industries Group Inc
Customer Address	1010 VD Parrott Jr Pkwy,
	Dalton, GA 10722, USA
Contact	Paul Murray
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Report Prepared by:	Angela Southey, PhD.



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Summary:

This major study confirms that airborne exposure to allergen and microbial particulates from carpets particularly when they are disturbed is surprisingly low. While true for both soiled used carpets and artificially contaminated new carpets, the observation is best demonstrated for the used carpets. Progressive disturbance appears to amplify the effect rather than lead to an increase in airborne levels.

One interpretation of these data is that particulate material is progressively driven toward the base of these carpets. When located at the base, it is likely to be more difficult to render it airborne. The data on sectioned carpets confirms that the majority of the allergen in a used carpet is found at the base and in new carpets with serial dust/allergen introduction a similar pattern becomes established following repeated disturbance.

The study demonstrates that an effective cleaning procedure beneficially impacts on both surface and airborne exposures to allergens and micro-organisms. As in an earlier study, the degree of airborne allergen exposure associated with artificially contaminated new carpets is low and is now demonstrated to be further reduced by the cleaning procedure. In the case of used carpets that are particularly soiled the levels of airborne exposure in the presence of disturbance were likewise low, again cleaning reduces them further. Taken together, these findings reinforce the belief that carpets can act as reservoirs for allergen, rendering it more difficult for these particles to become airborne. The findings also reinforce the desirability of regular carpet maintenance, with frequent vacuum cleaning and intermittent use of steam or water-based cleaning systems.

Future research addressing the factors, physical and otherwise, that impact on particle adherence to various carpet fibers will be highly informative, not least in establishing mechanisms that increase the reservoir capacity while at the same time allowing for effective removal during the various elements of a cleaning regime.



1. Test Item Description:

Six residential new carpets were submitted to AHG for testing. The carpets were an Unbranded N6 Broadloom, 'Full of Life' style #52N09 with a face weight of 25oz/yd.

Three of the carpets were treated with R2X and three were untreated with R2X but results were averaged as there was no discernible difference in results with or without this treatment.

3 residential used carpets, of similar or identical construction to the new carpets, were also submitted to AHG for testing from different areas of the USA. Refer to table below for the sample description and number of test runs completed for each carpet type.

Residential Carpets Sample Description	No. of test runs completed
New carpets 1-6	6
Used Carpet 1, Portland	1
Used Carpet 2, Las Vegas	1
Used Carpet 3, Chattanooga	1
Total Residential	10



2. Objectives:

The two main objectives of this study were to determine the following:

- i. The depth of penetration of various particulates in both new and used carpets.
- ii. The impact of cleaning on particulate removal at varying depths in both new and used carpets.

For each objective the approach, methodology, relevant results and conclusions are presented. In addition, all test methods and results that were generated during execution of this proposal are included in the report Appendices.



3. Background:

The data generated during Shaw #1 project on allergen bearing particle retention by carpets led to a conclusion that carpeting can and does act as a reservoir for particulate allergen. The observation is based upon data showing a reduction in airborne particulate levels during room disturbance in the presence of particular carpets.

While the data generated thus far is impressive, further data will be required to determine whether it is appropriate to change the negative attitude directed against carpets by the medical community. One important influencer is likely to be a clear-cut demonstration of the outcomes associated with defined cleaning procedures.

To date all Asthma and Allergy Friendly Certification protocols relating to textile based products that may act as allergen reservoirs, e.g. bedding and toys, incorporate a Care Code. It is likely that a similar Care Code would also be required in any protocol designed to consider potential carpet certification.

It is assumed that carpeting has a finite capacity to capture allergen bearing particles and will eventually become saturated. Beyond this point, the potential for increased airborne particle exposure becomes a concern. The data generated during Shaw #1 suggested that different carpet types may vary in this capacity. To address this concern, any Care Code developed must take account of these findings.

Prior to Care Code development, we must first establish a thorough understanding of exactly what is happening as carpets accumulate particulates, including allergen and micro-organisms, over time.



4. First Objective:

To determine the depth of penetration of various particulates in both new and used carpets.

Approach

To address this objective, it was necessary to determine the extent of particulate contamination of i) used 'naturally' contaminated carpets and compare them to ii) new artificially contaminated carpets.

The extent of particulate contamination of the used carpets was assessed by determining the concentrations of dust mite and cat allergens present in samples taken from the carpet surface prior to any intervention.

In the case of the new 'artificially' contaminated carpets, surface samples were taken after the introduction of allergen test dust (ATD), real household dust containing known concentrations of various allergens.

Microbial (bacteria and fungi) contamination present on the surface of the used carpets was quantified by surface swabs prior to any intervention. In the case of the new 'artificially' contaminated carpets, swabs were taken after introduction of micro-organisms (*S.epidermidis* and *A.niger*).

The effect of 4 successive room disturbances on the quantity/distribution of allergen and microbial contaminants on the carpet surface and in the air was measured for both used and new carpets.

Used and new artificially contaminated carpets were then sectioned into 3 layers to determine the depth of penetration of allergens before and after room disturbances.



- A number (n=3) of pre-selected used carpets, all more than 12 months old, were sourced by Shaw Inc. from different parts of the USA. Samples of dust had previously been collected from these carpets using mitest filters, as described in the Surface Allergen Sampling Procedure in Appendix 1, to ensure the presence of allergen contamination.
- 2. These carpets (3 in total) were cut, packaged and shipped to Airmid Healthgroup, as outlined in Appendix 2 to ensure that they remained upright and contamination from one section to another could not occur.
- 3. Six new carpets were artificially contaminated with allergen test dust (ATD) and with micro-organisms (*S.epidermidis* bacteria, *A.niger* fungi) as summarised in the New Carpet Testing outline in Appendix 4. This protocol has also been described in detail in the previous Shaw report.
- 4. All new and used carpets were studied individually in the environmental test chamber. Each carpet was laid in the chamber so that it completely covered the 11.4m² chamber floor area. Testing was conducted at 21°C ±3°C, 55% ±5% relative humidity (RH) with an air exchange rate of 1.0/hour. The flooring was allowed to equilibrate overnight under these conditions before testing commenced. Testing of the used carpets proceeded in the chamber as described in Appendix 3. Testing of the new carpets proceeded in the chamber as described in Appendix 4.
- 5. To determine the level of allergens and micro-organisms that could potentially become airborne during the investigation from the used and artificially contaminated new carpets, comparable measurements of airborne particles, allergen and micro-organisms were taken, using the air sampling procedures outlined in Appendix 5.
- 6. To determine the level of allergens and micro-organisms that were present on the surfaces of both used and artificially contaminated new carpets, comparable measurements of surface levels of allergen and micro-organisms were taken, using the appropriate surface sampling procedures outlined in Appendix 5.
- 7. Air and surface measurements were taken prior to any disturbance of the carpets, i.e. background and then after 4 successive room disturbances. The averages of these results are presented for the six new carpets, which were artificially contaminated with particulates (allergen and micro-organisms). The average results for Total particle counts for the 3 used carpets are presented. However, the results for airborne and surface



allergen are presented individually for the 3 used carpets. In the case of the airborne and surface microbial contamination, the results are shown for a single representative used carpet only. The data for the other two used carpets are presented in Appendix 8. It would have been misleading to average these data given the range of levels seen in each item.

- 8. The Results section contains graphs in the following figures:
- 9. Fig. 1 Average results for Total Airborne Particle Counts for Used (n=3) carpets
- 10. Fig. 2 Average results for Total Airborne Particle Counts for New (n=6) carpets
- 11. Fig. 3 Airborne dust mite and cat allergen levels for Used (n=3) carpets
- 12. Fig. 4 Airborne dust mite and cat allergen levels for New (n=6) carpets
- 13. Fig. 5 Surface dust mite and cat allergen for Used (n=3) carpets
- 14. Fig. 6 Surface dust mite and cat allergen for New (n=6) carpets
- 15. Fig. 7 Allergens in top, middle and base layers for Used (n=3) carpets
- 16. Fig. 8 Allergens in top, middle and base layers for New (n=6) carpets
- 17. Fig. 9 Airborne Micro-organisms for Used (n=1) carpet
- 18. Fig. 10 Airborne Micro-organisms for New (n=6) carpets
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- 20. Fig. 12 Surface Micro-organisms for New (n=6) carpets



Results:

Total Airborne Particle Counts

The graphs below show the average of the Total Airborne Particle Counts for the 3 used carpets (Fig. 1) and the 6 new carpets (Fig.2). Particle Counts were taken before and during 4 room disturbances (RD1-RD4). For the used carpets (Fig.1), RD1-RD4 was performed one after the other, allowing time for airborne particle counts to return to background levels between each room disturbance.

For the new artificially contaminated carpets (Fig.2), RD1 was performed with 1g/m² ATD on the carpet, RD2 was performed with 2g/m² ATD on the carpet and RD3 and RD4 were performed with 3g/m² and 4g/m² ATD on the carpet, respectively. The ATD was aerosolized into the chamber using a standard method. Room disturbances were not performed until the airborne particle counts had returned to background levels after the ATD introduction.

Interpretation:

As can be seen from Fig.1, the average total airborne particle counts for the used carpets, was remarkably low for background counts (entering the chamber) and remained low during the room disturbances. This was despite the large amount of dirt and debris which was visible on the surface of each carpet when they were laid in the chamber. As each additional room disturbance was preformed, there was minimal increase in the airborne particles, even during bouncing the ball.

In Fig.2, the average total airborne particle counts for the new carpets were higher during room disturbance than for the 3 used carpets (Fig.1). This indicates that either the artificially contaminated new carpets had a higher dust load than was found in the used carpets or that the particles were more adherent to or better retained by the fibers of the used carpets. Furthermore, Fig. 2 also shows that for the new carpets, the level of airborne particulates did not increase with each successive introduction of ATD and each successive room disturbance. This is similar to that data shown for used carpets in Fig. 1.









Airborne Allergen

The graphs below show the airborne allergen recovery for the 3 used carpets (Fig. 3A-3D) and the average airborne allergen recovery for the 6 new carpets (Fig. 4). For both used and new carpets, samples were taken at 1.5m and 0.75m heights during x 4 room disturbances (RD1-RD4). The graphs for the 1.5m height are shown in this section, the other graphs for the 0.75m height can be found in the Appendix.

For the used carpets, RD1-RD4 were performed once airborne particle counts had returned to background levels.

For the new artificially contaminated carpets (Fig.4), RD1 was performed with 1g/m² ATD on the carpet, RD2 was performed with 2g/m² ATD on the carpet and RD3 and RD4 were performed with 3g/m² and 4g/m² ATD on the carpet, respectively. Room disturbances were not performed until the airborne particle counts had returned to background levels after each ATD introduction.

Interpretation:

As can be seen from the used carpets in Fig.3A-3D, the airborne levels of dust mite and cat allergen were remarkably varied for the 3 different carpets, as would be expected due to varying degrees of contamination with dust mite and cat allergens in different homes.

'Portland Carpet'

In Fig. 3A 'Portland' carpet, dust mite allergen increased slightly at RD2 and RD3, as it was being released by disturbance but returned to background levels by RD4. These data would suggest that the room disturbance procedure increases the likelihood of allergen being retained by the carpet fibers, possibly as a result of it being driven down to the base level.

'Las Vegas' Carpet

The airborne dust mite allergen was much higher for the 'Las Vegas' carpet (Fig. 3B and 3C) than the Portland carpet (Fig 3A) at the initial background sampling. However levels decreased with each room disturbance to similar levels obtained for the Portland carpet. This might indicate that the disturbance protocol is having a compaction impact on allergenbearing particles within the carpet.

The background airborne cat allergen was very high (>1500pg) for the 'Las Vegas' carpet, much higher than the Portland and Chattanooga carpets tested in this study. Fig 3C shows that there was a decline in airborne cat allergen as each additional room disturbance was preformed after an initial peak during RD1. Although this carpet appeared to be saturated with cat allergen it was able to retain a substantial amount of the allergen after the peak at RD1 for the final three room disturbances. Again the room disturbance protocol may be resulting in a compaction effect on particles within the carpet.



'Chattanooga' Carpet

The 'Chattanooga' carpet in Fig. 3D had low levels of dust mite and cat allergen but these increased to a peak at RD3 and then decreased at RD4. This followed a similar pattern to the Portland carpet in Fig. 3A.

As discussed earlier, total airborne particle counts $(0.3-10\mu m)$ for the used carpets did not increase after each additional room disturbance, despite the changes observed in airborne allergens. For example no increase in counts was observed at $3\mu m$ (corresponding to airborne cat allergen) for the Las Vegas carpet during RD1. It must be remembered that the airborne allergen is picogram amounts ($1pg = 1x \ 10^{-12}g$) so the levels of allergen-associated particles may be too low to be detected by the laser particle counter.

There is a need for further work assessing the correlation between pictogram quantities of allergen/m³ and low levels of comparably sized particles, as measured by laser diffraction systems.

New carpets

In Fig.4, the average airborne allergen for the new carpets showed an initial peak at RD1 and then a decrease at RD2. The levels of dust mite and cat allergen then increased in a stepwise manner from RD2 to RD4, as each additional 1g/m² ATD was introduced into the chamber. The artificially contaminated new carpets were likely to be saturated with ATD leading to the stepwise increase observed in airborne allergens with RD2 to RD4. Fig. 2 shows that for the new carpets, like the used carpets, levels of total airborne particulates did not increase with each successive introduction of ATD and room disturbance, even though airborne allergen levels fluctuated.





Fig. 3B



Fig. 3C – SCALE IS 5x HIGHER THAN Fig. 3B -Showing actual levels of cat allergen recovered













The graphs below show recovery of surface allergens, dust mite and cat, from each of the 3 used carpets (Fig. 5A-5D) and the average surface allergen recovery for the 6 new artificially contaminated carpets (Fig. 6). For used carpets, mitest samples were taken after each room disturbance was completed. In the case of the new carpets, mitest samples were also taken after each room disturbance RD1-RD4. It should be noted that the quantity of ATD on the new carpets was $1g/m^2$ for RD1, $2g/m^2$ for RD2, $3g/m^2$ for RD3 and $4g/m^2$ for RD4.

Interpretation:

Fig.5A to 5D show that the quantity of dust mite and cat allergens recovered from each of the 3 used carpets was very different.

'Portland' carpet

The 'Portland' carpet (fig. 5A) had approximately 10-20ug/m² of dust mite allergen recovered from the surface after room disturbances. For additional room disturbances performed there was a small increase in dust mite allergen levels. This was mirrored in the airborne allergen results with dust mite increasing slightly after each room disturbance up to RD3.

'Las Vegas' Carpet

The 'Las Vegas' carpet (Fig. 5B) had markedly high levels of cat allergen and no detectable dust mite allergen. (Note: 3 cats were living in this apartment). Fig 5C shows this carpet on a 10x larger scale, the levels of cat allergen recovered were as high as 900ug/m² after RD1 and decreased to approx. 400ug/m² for the remaining 3 room disturbances. A peak in airborne cat allergen was also seen during RD1 for this carpet which then levelled out over the next 3 room disturbances.

'Chattanooga' carpet

In contrast to the Las Vegas carpet, >100ug/m² of dust mite allergen were found in the 'Chattanooga' carpet after RD1 and RD2 and very little cat allergen. The dust mite allergen decreased to <20ug/m² after the next 2 room disturbances. This carpet had relatively low levels of airborne dust mite, despite the high surface concentrations obtained after RD1 and RD2 indicating allergen retention.

The observed decreases in quantity of allergen recovered for the used carpets, after RD1/RD2, may be due to the allergens being gradually pushed down towards the base of the carpet rendering them unavailable for surface (and airborne) recovery.

New carpets

Fig.6 shows the average surface dust mite and cat allergen recovered from the new carpets (n=6). The graph shows that with each successive room disturbance, there was a stepwise



increase of dust mite and cat allergen recovered as more ATD was introduced. This trend levelled out slightly after RD4 for dust mite allergen recovery. A similar pattern was seen for the airborne allergens measured during RD1-RD4 for these carpets.



20 0

Background

RD1





RD2

RD3

RD4











Depth of penetration of allergen in the Top, Middle and Base layers

of carpets

To determine the depth of penetration of dust mite and cat allergen in the top, middle and base layers of 3 used carpets and 6 new carpets, the top two layers (approx. 5mm height) of the carpets were cut as per protocol in Appendix 7. The cut carpet fibers were harvested and extracted along with the carpet bases and analysed for dust mite and cat allergen content. Results were expressed in pg/mm³ of carpet, based on the surface area of the carpet that was cut and sampled. The graphs below show recovery of dust mite and cat allergens from the top, middle and base layers of the 3 used carpets at background, after RD1 and after RD4 (Fig. 7A-7D). The carpet segments were removed after each room disturbance was completed.

'Portland' carpet

Fig 7A shows the 3 layers of the used 'Portland' carpet. More cat allergen was recovered in the top and base layers than the middle layer of this carpet. Similar quantities of cat allergen (pg/mm³) were detected in the base layers, though varying levels were found in the top layer at each test stage. The cat allergen may have been unevenly distributed over the top of the carpet, which would explain variation in the results obtained in the top layers of the carpet segments sampled. A significant amount of dust mite allergen was recovered from the base layers, increasing between RD1 to RD4, but very little from the top and middle layers.

'Las Vegas' Carpet

Fig 7B and 7C show the recovery of cat allergen from the used 'Las Vegas' carpets. As observed with the airborne and surface allergen data, no dust mite was detected in the carpet segment layers, while very high levels of cat allergen were measured. Again the scale of the graph had to be adjusted (100x) to give a clear picture of the amount of cat allergen actually present. What's interesting from Fig. 7C is that the majority of the cat allergen was detected in the base layers at background and following RD1 and RD4.

'Chattanooga' Carpet

In Fig. 7D, the 'Chattanooga' carpet was similar to the 'Portland' carpet in terms of penetration of dust mite to the base layers and how it increased gradually from RD1-RD4. While some cat allergen was found in the base of this carpet very little was obtained in the other 2 layers sampled. Like the other used carpets, these results correspond to the surface and airborne allergen levels detected in the used Chattanooga carpet.

New Carpets

Fig. 8 shows the average results for the 6 new artificially contaminated carpets at background, RD1 and RD4. The results show that the ATD needed to be at least 4g/m2 (i.e. at RD4) in order to obtain detectable levels/penetration of dust mite and cat allergen in the



top, middle and base layers. At this level of allergen loading, the majority of the allergens are found in the base layers, like the used carpets but to a lesser extent. The lower levels of allergens found in the artificially contaminated carpets may not only be due to differences in allergen load but also the fact the used carpets may be able to retain more allergen in the base layer as they are become worn, soiled and undergo alteration in fiber-particle adherence properties through daily use in the home.





















Airborne Micro-organisms

The graphs below show the airborne micro-organism recovery for 1 used carpet (Portland, Fig. 9) and the average airborne micro-organism recovery for the 6 new carpets (Fig. 10). For both used and new carpets, samples were taken at 1.0m height during room disturbance.

For the new artificially contaminated carpets RD5 was performed after bioaerosols (*S.epidermidis* and *A.niger*) were introduced onto the ATD-loaded carpets.

Interpretation:

In Fig. 9, the naturally contaminated used 'Portland' carpet had undetectable levels of airborne fungi and bacteria in the initial background sampling prior to room disturbance. However when the carpet was disturbed a number of times, an increase in airborne bacteria was obtained, which decreased again by RD4. Some airborne fungi were initially obtained after RD1, this decreased to background at RD3. The changes in airborne micro-organisms over the room disturbances may indicate that they were initially being brought to the surface.

As can be seen from Fig. 10, low levels of airborne fungi and bacteria were detected in the background samples of the 6 artificially contaminated new carpets prior to bioaerosol introduction likely associated with micro-organism contamination of ATD.

Following introduction and during room disturbance, high levels of fungi and bacteria were detected in the air samples of these new carpets. In comparison to the used carpets, artificially contaminated carpets had much higher levels of airborne micro-organisms during room disturbance. The quantity of fungi and bacteria introduced to the new carpets was markedly higher than that recovered from the used carpets which were naturally contaminated over time in the home.







Surface Micro-organisms

The graphs below show the surface micro-organism recovery for 1 used carpet (Portland, Fig. 11) and the average surface micro-organism recovery for the 6 new carpets (Fig. 12). For the used carpets, swab samples were taken from the carpet surface after each of 4 room disturbances.

For the new artificially contaminated carpets swabs were taken after RD5, which was performed after the bioaerosols (*S.epidermidis* and *A.niger*) were introduced onto the ATD-loaded carpets.

Interpretation

As for the airborne micro-organisms (Fig. 9 and 10), there was a large difference in the quantity of fungi and bacteria recovered from the used naturally contaminated carpet compared to the artificially contaminated new carpets. In the 'Portland' used carpet (Fig. 11), similar quantities of bacteria (approx. $50x10^3$ cfu/m²) were recovered after each disturbance but very little fungi. Whereas the new carpets in Fig. 12 up to 400×10^3 cfu/m² of bacteria were recovered in the swabs during RD5, after introduction of *S.epidermidis*. Detectable levels of *A.niger* spores ($70x10^3$ cfu/m²) were also recovered from the new carpets during RD5.









^{*}For new carpets, only 1 room disturbance was performed after the introduction of micro-organisms and prior to cleaning.



To determine the impact of cleaning on particulate removal at varying depths in both new and used carpets.

Approach

Having determined the extent of particulate presence in the used 'naturally' contaminated carpets and the new 'artificially' contaminated carpets, the second objective was to examine the effect of cleaning.

The cleaning process was assessed in terms of the quantity/distribution of particles, allergen and microbial contaminants on the carpet surface and in the air.

Used carpets and new artificially contaminated carpets were then sectioned into 3 layers to determine the depth of penetration of the dust mite and cat allergens after implementation of the cleaning protocol.

Methodology:

- 1.1 Following assessment of the used carpets and the new 'artificially' contaminated carpets in the first objective, the cleaning process was carried out as described in Appendix 6.
- 1.2 To determine the level of allergens and micro-organisms that could potentially become airborne during and after the cleaning process, comparable measurements of airborne particles, allergen and micro-organisms were taken, using the air sampling procedures outlined in Appendix 5.
- 1.3 To determine the level of allergens and micro-organisms that were present on the carpet surfaces after the cleaning process, comparable measurements of surface allergen and micro-organisms were undertaken, using the appropriate surface sampling procedures outlined in Appendix 5.
- 1.4 The average results are presented for the cleaning of the six new carpets, which were artificially contaminated with particulates (allergen and micro-organisms). The average airborne particle counts, during and after cleaning, are presented for the 3 used carpets. The effect of cleaning on airborne and surface allergen shown individually for the 3 used carpets. In the case of the airborne and surface microbial contamination, the results are shown for a single representative used carpet only. The data for the other two used



carpets are presented in Appendix 8. These data could not be averaged given the range of levels seen in each item.



Results:

Total Airborne Particle Counts

The graphs below show the average of the Total Airborne Particle Counts for the 3 used carpets (Fig. 13) and the 6 new carpets (Fig.14) during room disturbances before and after the cleaning process.

Interpretation

The impact of the cleaning procedure on surface and airborne allergen levels was highly significant for the new and artificially contaminated carpets. Essentially these levels were reduced to almost zero.

By contrast, and as shown above, airborne particle counts associated with disturbance were minimally elevated for the used carpets. Cleaning probably does reduce levels but there is hardly any potential to demonstrate a meaningful effect.





Fig. 14





Airborne Allergen

The graphs below show the Airborne Allergen levels measured at 1.5m height for the 3 used carpets (Fig. 15A-15C) and the 6 new carpets (Fig.16) during room disturbances before, during and after the cleaning process.

Interpretation

It is clear that, where airborne allergen can be measured, a meaningful reduction in levels is achieved after the cleaning process is completed. This interpretation is best demonstrated by cat allergen levels in the 'Las Vegas' carpet, and probably also for dust mite in this carpet. Indeed airborne dust mite levels are reduced by cleaning for all 3 carpets tested. Furthermore, there is a small but definite reduction in airborne cat allergen levels for the Chattanooga carpet.

In the case of the new carpets (n=6), there is a very meaningful reduction airborne allergen levels as a consequence of room disturbance before and after cleaning. This observation is applicable for both allergens tested, dust mite and cat allergen.

In summary these data would suggest that when allergen is present in a carpet of this type, cleaning can have a strong impact on the reduction of airborne allergen levels, and by extrapolation, exposures for people living in homes.











Surface Allergen

The graphs below show the recovery of Surface Allergen for the 3 used carpets (Fig. 17A-17D) and the average recovery of surface allergen for the 6 new carpets (Fig.18) at the end of the room disturbances carried out before and after the cleaning process.

Interpretation

Surface allergen levels were very markedly reduced by the cleaning procedure on the new residential carpets. This was demonstrable for both allergens tested and the differences were the greatest observed throughout the whole carpet cleaning stage of these studies.

Dust mite allergen levels in the used Chattanooga carpet and the used Portland carpet were markedly reduced. The impact appears to be less than it was given the scale on the ordinate of the graph. Again, cat allergen levels in the 'Las Vegas' carpet, known to be extremely high, were greatly reduced by cleaning (please note it was necessary to increase the ordinate scale here).



Fig. 17A
























Depth of penetration of allergen in the Top, Middle and Base layers of cleaned carpets

To determine the depth of penetration of dust mite and cat allergen in the top, middle and base layers of 3 used carpets and 6 new carpets after the cleaning process. Results were expressed in pg/mm³ of carpet, based on the surface area of the carpet that was cut and sampled.

The graphs below show recovery of dust mite and cat allergens from the top, middle and base layers of the 3 used carpets (Fig. 19A-19D) and the average of 6 new carpets (Fig. 20) before and after cleaning.

Interpretation

In the case of new residential carpets, the most striking finding is the persistence of dust mite and cat allergens at the base of the carpet following cleaning. While admittedly there is a marked reduction, it is essential that a removal method be established to address the persistence of this allergen reservoir in spite of intervention.

Scanning electron microscopy (SEM) clearly demonstrates the persistence of 'dirt' clumps and allergen bearing particles among others, at the base of the carpet following the cleaning procedure. The SEM prints are shown together in Appendix 9.

In the case of the used carpets, the same phenomenon is demonstrable for two of the three, namely the 'Portland' and the 'Las Vegas' carpets. In the case of the 'Portland' carpet both allergens were demonstrable at the base. The 'Las Vegas' carpet, with its very high cat allergen levels, retained unacceptably high contamination levels following the cleaning procedure. Again the SEM prints confirm the persistence of unacceptable contamination (Appendix 9).













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Airborne Micro-organisms

The graphs below show the airborne micro-organism recovery before and after the cleaning process for 1 used carpet (Portland, Fig. 21) and the average airborne micro-organism recovery for the 6 new carpets (Fig. 22). For both used and new carpets, airborne micro-organisms were sampled at 1.0m height during room disturbances which were carried out just before and after cleaning.

Interpretation:

The impact of cleaning on airborne micro-organism levels from new residential carpets is impressive. This is evident both for bacterial and fungal organisms.

Again, the potential for cleaning to reduce airborne micro-organism levels, in this case from the used carpets, is demonstrable. Data for the Portland carpet for both groups of micro-organisms shows a reduction, again the impact somewhat under-represented by the scale of the graph.











Surface Micro-organisms

The graphs below show the surface micro-organism recovery before and after the cleaning process for 1 used carpet (Portland, Fig. 23) and the average surface micro-organism recovery for the 6 new carpets (Fig. 24). For both used and new carpets, surface micro-organisms were sampled after room disturbances which were carried out before and after cleaning.

Interpretation:

The impact of cleaning on surface micro-organism levels on new residential carpets is impressive. This is evident both for bacteria and fungal organisms.

Again, the potential for cleaning to reduce surface micro-organism levels, in this case on the surface of used carpets, is demonstrable. Data for the Portland carpet for bacterial counts shows a reduction, again the impact is somewhat under-represented by the scale of the graph.













Appendix 1: Surface Allergen Sampling Procedure

Mitest filters to allow for the rapid collection of dust from a surface when the filter is placed inside the dust collector and attached to the nozzle of a vacuum cleaner.



Mitest Dust Collector **Mitest Filter**

Allergen Sampling Procedure:

- 1) Select the desired sampling location on the carpet, preferably a flat area away from foot fall.
- 2) Mark out an area on the carpet equivalent to the size of an A4 page (approx. 0.06 m^2)
- 3) Remove the Mitest dust collector from the sealed packet and insert the plastic Mitest filter into the Mitest Dust collector nozzle
- 4) Ensure that the open end of the filter is facing upwards.
- 5) Slot the dust collector nozzle and filter onto the top of the vacuum cleaner wand. Once the vacuum is turned on, the suction from the vacuum cleaner will hold the filter in place.
- 6) Switch on the vacuum cleaner at full power.
- 7) Vacuum within the marked area in a series of horizontal and vertical strokes for 2 minutes.



- 8) For maximum dust recovery, ensure that the person carrying out the sampling applies pressure to the area during vacuuming.
- 9) Switch off the vacuum cleaner after 2 minutes has elapsed.
- 10) Carefully remove the Mitest filter from the dust collector and place in the labelled tube provided.
- 11) Record the sample date, sample number and location of carpet on the label.
- 12) Sampling should be carried out in duplicate for each carpet
- 13) In case of used carpets being sampled in the USA, submit the appropriate carpet samples to Airmid Healthgroup- Allergen laboratory for testing.

Appendix 2: Harvesting of Used Carpets from Homes to ship to AHG Laboratory

- Select used carpets from rooms that are a suitable size (e.g. 12ft x12ft) to be laid in the test chamber without any gaps by the walls.
- 2) Take mitest samples and send to lab to confirm that it contains significant levels of dust mite and/or cat allergen, as per mitest procedure in Appendix I.
- 3) Selected carpets with suitable levels of allergen will be removed as follows:
 - 6ft (2m) x 3ft (1m) rectangles are marked on the carpet with a permanent marker pen using the example shown. Each section is labelled with room number A, B or C followed by a sequential number e.g. from 1 to 16.

•	1A	•	2A	•	3A	•	4A
•	5A	•	6A	•	7A	•	8A
•	9A	•	10A	•	11A	•	12A
•	13a	•	14A	•	15A	•	16A

Example of Mark-up of a Carpet:

- 2. The carpet segments are carefully lifted and placed in layers in a cardboard box of the same dimensions (6ft x 3ft). A layer of lining paper or polythene sheet is placed between each segment of carpet. The carpet must be kept upright so as to preserve the allergen and microbial load within the carpet fibres.
- 3. A label indicating that it must be kept UPRIGHT is written on each box.



- 4. The box is sealed and placed on a pallet and wrapped with pallet wrap for shipment to the laboratory. Upon receipt at the laboratory the carpet is logged into the Sample receipt database.
- 5. The carpet is laid in the environmental test chamber in the same order as it was harvested, as shown in the mark-up above. This is to try to keep the allergen and microbes where they originally were in the house.

Appendix 3:

Used Carpet Testing Outline

Each carpet was disturbed by 4 successive room disturbances and then subjected to the carpet cleaning protocol. Air and surface samples were taken for particle sizes, allergen and for micro-organisms (Total Viable Counts for bacteria and fungi) during room disturbance and during cleaning. The cleaning protocol (Appendix 6) then commenced followed by a final room disturbance after the carpet had dried.





Appendix 4

New Carpet Testing Outline

4.1 Each new carpet (with or without R2X) was tested in triplicate in the chamber in the following manner. Each carpet was sequentially loaded with ATD (1g/m² per loading x4), micro-organisms (*S.epidermidis* and *A.niger* bioaerosols) and then subjected to room disturbances. This was followed by the carpet cleaning protocol. Air and surface samples were taken for particle sizes, allergen and for micro-organisms during room disturbance and during cleaning.



4.2 On the first day of testing each new carpet, Allergen Test dust (ATD), containing cat allergen and dust mite allergen was introduced into the chamber. The ATD was aerosolized into the chamber via an entry port using compressed air @ >50psi and distributed using a mounted ceiling fan. 12g of ATD was introduced at each of 4 introductions over 1 day giving a final ATD concentration of 4g/m². Between each introduction, the room was disturbed by 5min walking and 5min bouncing a basketball. On day 2 the micro-organisms were introduced, followed by a room disturbance. The cleaning protocol (Appendix 6) then commenced followed by a final room disturbance after the carpet had dried.

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Appendix 5 – General Methodology for Sampling Air and Surfaces

5.1 Air Sampling

Airborne particles ranging in size from 0.3 to 10μ m were counted at 1min intervals using a Met One Laser diffraction particle counter at each stage of the testing outlines in Appendix 3 and 4.

Airborne Micro-organisms were collected for 5min duration onto a biostage impactor, operated under vacuum at 28.5L/min. The air samples were collected onto 2 selective agars specifically for the growth of fungi and bacteria.

Airborne allergen samples were collected onto filter cassettes (at 0.75m and 1.5m heights) using Side Kick pumps (2L/min). Samples were analysed for dust mite and cat allergen by augmented ELISA.

5.2 Surface Sampling

Duplicate swabs were taken as follows: A 10x10cm template was randomly placed on the carpet and a swab was used to sample the surface of the carpet. The swab was soaked in PBS. Samples were kept on ice until processing in the lab. Samples were analysed for Total Viable Counts (TVC) of bacteria and of fungi by serially diluting the samples onto selective media and incubating at appropriate conditions.

Surface allergen samples $(0.06m^2)$ were taken in duplicate using mitestTM filters after each stage of the carpet testing outlines in Appendix 3 and 4. Refer to Appendix 1 for surface allergen sampling procedure. Samples were analysed for Dust Mite allergen (*Der p1*) and Cat allergen (*Fel d1*) by allergen-specific ELISA.



Appendix 6: Outline of Residential Carpet Cleaning Protocol

New and used Carpets were tested in batches of three, as described above. After room disturbances, carpets were treated using the cleaning protocol for residential carpets provided by Shaw. The table below summarises how the cleaning protocol was performed in the chamber. During the cleaning process, particle counting was performed along with air sampling for allergen and micro-organisms. Following cleaning, the carpets were subjected to a final room disturbance and the carpets were surface-sampled for allergen, micro-organisms and further SEM analysis.

RESIDENTIAL CARPET CLEANING SUMMARY

- BACKGROUND COUNTS 5min
- VACUUM ON, NOT MOVING, 5min
- VACUUMING 2 PASSES Collect Air Samples 10min
- SPRAY CARPET WITH CLEANING AGENT 5min
- BRUSH CARPET 5min
- WAIT 10min FOR CLEANING SOLUTION TO WORK
- SPRAY CARPET WITH WARM WATER Collect Air Samples 10min
- APPLY SUCTION TO CARPET 5min
- ALLOW TO DRY OVERNIGHT WITH FAN ON.



Appendix 7: Sectioning Carpets for Allergen Analysis (Top, Middle and Based Layers)

At various stages of the testing protocol, a carpet segment (0.06m²) was cut and removed from the chamber. The segment was labelled and stored frozen until sectioning commenced. The segment was dissected into three different layers – the upper layer, middle layer and bottom layer. Each layer was analysed for the presence of dust mite allergen and cat allergen. A scanning electron micrograph (SEM) was also taken of a select number of carpets, pre and post treatments.

Details:

- 1. A 0.06m² segment of carpet is removed from the chamber at various stages of testing protocol. The segment can be replaced with a clean carpet segment,
- 2. Two horizontal layers, each of 5mm height, are cut from the carpet segment
- 3. The samples that are cut are called 'Top Layer', 'Middle Layer' and the base part of the carpet after the two layers have been removed is called 'Base'.
- 4. The carpet shearer must only be used by a qualified machine operator.
- 5. Check the blades on the carpet shearer are clean.
- 6. Set the blade cutting height to 5mm.
- 7. Place the carpet segment in the cabinet and cut with one stroke along the entire length of the segment.
- 8. Collect all sheared carpet and dust using a mitest filter and vacuum cleaner. Normally 3-4 mitests are filled during this procedure.
- 9. Place mitest filters into the labelled tubes.
- 10. Wipe blades with a wipe and cut the next layer or the next carpet segment and so on.
- 11. Store top and middle layer samples and bases in fridge until analysis.
- 12. Extract each mitest with 15ml of PBS-Tween and extract the carpet bases with 400ml of of PBS-Tween.
- 13. Analyse for dust mite and cat allergens, calculate the amount of allergen in pg/mm³ of carpet.



Shaw 2 Final Report- Residential Carpets Appendix 8

Additional Results for New Residential Carpets

This section is presented as follows:

- Average Airborne Particle Counts showing individual particle sizes (0.3-10micron) during x 4 room disturbances
- 2) Average Airborne Particle Counts obtained during Cleaning
- 3) Average Airborne Allergen recovery at 0.75m during x4 RD
- 4) Average Airborne Allergen recovery at 0.75m during and after cleaning



1) This graph shows the Average Airborne Particle Counts obtained for 6 new residential carpets during 4 room disturbances.



Note: $1g/m^2$ of ATD was added between each room disturbance.



2) These graphs show the Average Airborne Particle Counts obtained for 6 new residential carpets during and after cleaning.



Note: Y-axis Scale is 5x higher than for used carpets during cleaning.





3) This graph shows the Average Airborne Allergen Levels obtained for 6 new residential carpets during x4 RD. Air samples were taken at 0.75m height.



4) This graph shows the Average Airborne Allergen Levels obtained for 6 new residential carpets before, during and after cleaning. Air samples were taken at 0.75m height.





Additional Results for Used Residential Carpets

Used carpet 1	Chattanooga		
Used carpet 2	Portland		
Used carpet 3	Las Vegas		

This section is presented as follows:

- 1. Airborne Particle Counts showing individual particle sizes (0.3-10micron) during x 4 room disturbances for each of the 3 used residential carpets.
- 2. Airborne Particle Counts obtained during and after Cleaning for each of the 3 used residential carpets. Individual particle sizes (0.3-10micron) are shown.
- 3. Airborne Allergen recovery at 0.75m during x4 RD for each of the 3 used residential carpets.
- 4. Airborne Allergen recovery at 0.75m during and after cleaning for each of the 3 used residential carpets.
- 5. Recovery of Surface micro-organisms (TVC) after x 4 RD and after cleaning for 2 used residential carpets (Las Vegas and Chattanooga).
- 6. Recovery of Airborne micro-organisms (TVC) after x 4 RD and after cleaning for 2 used residential carpets (Las Vegas and Chattanooga)



1) These graphs show the Airborne Particle Counts obtained for 3 used residential



carpets during 4 room disturbances









2) These graphs show the Airborne Particle Counts obtained for 3 used residential carpets during and after cleaning.



















3) These graphs show the Airborne Allergen Levels obtained for 3 used residential

carpets during x4 RD. Air samples were taken at 0.75m height.







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4) These graphs show the Airborne Allergen Levels obtained for 3 used residential

carpets before, during and after cleaning. Air samples were taken at 0.75m height.









5) These graphs show the recovery of surface micro-organisms (TVC) after 4 room









6) The graphs below show the recovery of airborne micro-organisms (TVC) after 4





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Shaw 2 SEM Report - of Residential Carpets





Appendix 9 - Scanning Electron Microscopy (SEM) Images of Carpets

A 1cm² piece of carpet was cut from the carpet segment which was to be used for sectioning, as outlined above.

A total of 3×1 cm² pieces were taken from each carpet at the following test stages:

- a) background, prior to any intervention in the chamber
- b) after 4 ATD and 1 bioaerosol introduction i.e. fully loaded carpet
- c) after cleaning and drying overnight

The small sample was placed in a labelled container and sent to the Centre for Microscopy and Analysis in Trinity College Dublin for scanning electron microscopy (SEM imaging). The sections were imaged a 200x.

Images from 4 new residential carpets and 2 used residential carpets (Portland and Chattanooga) are shown in this report.

As well as providing information as to the allergen and micro-organism content of the carpet, the SEM also provided information as to the conformational differences between new and used carpets, and the particle/fiber adherence.



Appendix 9- New Residential Carpet Sample 1 (ASC002071-1)



Interpretation:

This is an example of the SEMs obtained for one of the new residential carpets at background, before contamination with ATD, after x4 ATD & 1 bioerosol introduction and after cleaning.

At background (left panel) there is some contamination with dust particles, which may have accumulated on the carpet during storage, prior to testing.

In the middle panel, there is obviously more dust, particularly on the top layer, due to the presence of ATD and microbial contaminants.

In the right panel, there is obviously less dust on the top and middle layer, due to removal by the cleaning process. There is still some observed at the base, which correlates with Fig. 20 in the main report. Fig. 20 shows some penetration of allergens into the base of the carpet, even after cleaning.



Appendix 9- New Residential Carpet Sample 2 (ASC002071-2)



Interpretation:

This is another example of the SEMs obtained for one of the new residential carpets at background, before contamination with ATD, after x4 ATD & 1 bioerosol introduction and after cleaning.

At background (left panel) there is very little contamination with dust particles. In the middle panel, there is obviously more dust, particularly on the top layer, due to ATD and microbial contamination. There is also some in the middle and base layers, possibly redistributed from the top after the x4 room disturbances.

In the right panel, some dust particles can be seen in the top and middle layer, which remained after the cleaning process. There is a trace amount at the base of this carpet.







Interpretation:

The results obtained for this carpet are very similar to the previous two new carpets, please refer to observations above.



Appendix 9- New Residential Carpet Sample 3 (ASC002099-2)



Interpretation:

The results obtained for this carpet are very similar to the previous new carpets, please refer to observations above. <u>Note</u>: in the right panel of images, the base layer has a marked amount of dust contamination after the cleaning process.


Appendix 9- Used Residential Carpet (Portland)



Interpretation:

Here are the SEMs obtained for the used residential Portland carpet at background, after x4 room disturbances and after cleaning.

At background (left panel) there is a high level of contamination with dust particles, which may have accumulated in the carpet fibers over time in the home. Note that the distribution of the contamination is throughout the top, middle and base layers.

In the middle panel the images are similar to the background after the x4 room disturbances but there seems to be more dust in the base layer here.

In the right panel, there is slightly less dust on the top layer and base layer, due to removal by the cleaning process. There is still a considerable quantity of dust contaminants observed in the middle layer, which correlates with Fig. 19A in the main report for this carpet. Fig. 19A shows the presence of allergens particularly in the base of the carpet, even after cleaning.



Appendix 9- Used Residential Carpet (Chattanooga)



Interpretation:

Here are the SEMs obtained for the used residential Chattanooga carpet at background, after x4 room disturbances and after cleaning.

At background (left panel) there is some contamination with dust particles, but to a lesser degree than for the Portland carpet. The distribution of the contamination is throughout the top, middle and base layers.

In the middle panel the SEM images the top and base layer are similar to the background images. However there seems to be more dust in the middle layer possibly due to redistributing of the dust by room disturbances.

In the right panel, there is notably less dust on the top, middle and base layers, due to removal by the cleaning process. This correlates with Fig. 19D in the main report for this carpet.