

Causal inference and the design of clinical trials in  
the community environment: a pilot study of  
allergen-reduction for the amelioration of childhood  
asthma.

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“How can you prove that it is true, that a causal association exists, between two complex multifactorial organic factors?”

- Brent Caldwell

“Pilate saith unto him, What is truth?”

- Pontius Pilate (John, chapter 18, verse 38, King James Version of the Bible)

“Beauty is truth, truth beauty," - that is all

Ye know on earth, and all ye need to know.”

- John Keats (from “Ode on a Grecian Urn”)

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## **Abstract**

This thesis tested the hypothesis that it was possible to successfully adapt to the New Zealand community setting, the study design of an American allergen-reduction trial. The American study reported by Morgan and colleagues translated the gold-standard scientific method for testing causal associations, so that it could be taken from the ideal world of the laboratory, and applied in the ‘real life’ American community domestic environment. This thesis elucidates the key components of the scientific proof of a causal association, and outlines the issues involved in the adaptation of the American study in order to incorporate these key components, and take account of the relevant differences between America and New Zealand (such as, the kinds of allergens, nature of domestic houses, and cultural differences). The major adaptation was the development of placebo interventions because Morgan and colleagues did not take account of the placebo effect.

A systematic review of allergen-reduction trials in childhood asthma is presented, and an assessment is made of the degree to which the key components of the scientific proof of causality were able to be included without compromising their integrity, in both the New Zealand adaptation, and similar studies reported in the literature. The thesis concludes that because of flaws in the designs of research performed to date (such as absence of a control group, or lack of a placebo, or inadequate randomisation protocols) there is insufficient evidence for or against the allergen-reduction hypothesis. This thesis makes a contribution by outlining the key study-design components that any future study must possess in order to scientifically test the allergen-reduction hypothesis.

In the process of reviewing the literature, and critically analysing it, it became apparent that it was necessary to take a step back, to take a wider view of concepts and assumptions that lie prior to, and underpin, the American study and allergen-reduction research in general. This thesis explores logic and causality and their role in scientific studies of allergen-reduction, and points to reasons why research has been unable to provide definitive answers, and identifies the key features that any future study must possess, in order for it to conclusively accept or reject the allergen-reduction hypothesis once and for all. Research in this field to date has paid too little attention to theory, and this thesis makes a contribution by explicating the relevant theories of logic, causality, and immunology which must inform the design of a study if it is to have any chance of delivering interpretable and useful results.

The outcomes of the pilot study of the New Zealand adaption of the study design of Morgan and colleagues are outlined, along with a critical discussion about the lessons learned from the pilot.

The thesis concludes that extensive changes are needed to the pilot study design in order for it to have scientific validity, and to ensure it is acceptable to the study-participants and to the asthmatic children in the community to whom the results of the study should be applied.

## 0 Introduction

The hypothesis of this thesis was that allergen-reduction interventions, that had been shown to be effective in an American study, could be effectively modified and deployed in the New Zealand community setting, in a manner that was acceptable to the participants, and which promoted a high degree of compliance and scientific rigour. This thesis is divided into four main parts: a methodological review of community trials; a systematic review of allergen-reduction interventions to improve childhood asthma; a description and analysis of a pilot study of an adaptation of the study by Morgan and colleagues, and a discussion of what kind of study design would be required in any future trials so that they can advance knowledge in this area.

### *What is asthma?*

Asthma is a spectrum of symptoms, characterised by recurrent wheeze and/or cough due to intermittent reversible obstruction of airflow and increased bronchial hyperresponsiveness, caused by inflammation in the bronchi [1, 2]. Typically studies define asthma as the presence of wheeze, dyspnoea and prescription of inhaled steroids [3], and use questionnaires with multiple items and a continuous asthma symptom scale to increase their positive predictive value [4]. The diagnosis of asthma is particularly problematic in young children because they commonly have transient asthma symptoms when they have respiratory infections that completely resolve when the infection has gone [5].

### *Intrinsic versus extrinsic, atopy, and allergens*

Traditionally asthma has been divided into two types according to whether there is an identifiable outside trigger of bronchoconstriction (extrinsic asthma) or if no such external trigger can be found (intrinsic asthma). External factors that can trigger bronchoconstriction include allergens, cold air, strong smells, to name a few. Allergens are molecules that bind to components of the immune system (particularly type 2 T-lymphocytes) which causes a chain of chemical reactions which, in the airway, leads ultimately to bronchoconstriction and airflow obstruction [6]. People who react in this way to allergens are defined as being ‘atopic’ and are classified as having ‘extrinsic asthma.’ However, even people with intrinsic asthma, have increased bronchial hyper-responsiveness to non-specific causes, such as methacholine [7].

Atopy is important because atopic asthmatics, who are exposed to allergens, experience worsened asthma and asthmatic children who are also atopic are more likely to continue to have chronic asthma that persists into their adult lives, and atopic asthmatics who have bronchial hyper-responsiveness tend to have more severe asthma [8]. Hence it is reasonable to hypothesise that reductions in allergen exposure would lead to reduced severity of asthma for atopic asthmatics.

However, there is some uncertainty in the science underpinning this allergen-reduction hypothesis:

1. the division of asthma into two separate categories of extrinsic versus intrinsic asthma has been challenged, because several components of the ‘extrinsic

asthma' biochemical pathway have been found to be present in non-atopic 'intrinsic' asthmatics [2, 9];

2. skin prick tests (SPTs), which are used as to define atopy, do not have perfect positive or negative predictive values for the identification of sensitivity to inhaling allergens [10], or to hyper-responsiveness to methacholine [7];
3. it is possible that Immunoglobulin E (IgE) is the cause of worsened asthma because even in non-atopic (negative skin prick test, no history of atopy, normal specific IgE levels) asthmatics, those who have total IgE levels  $>150\text{Uml}^{-1}$  have more severe asthma than non-atopic asthmatics with lower total IgE levels [2].
4. the value of differentiating atopic from non-atopic asthmatics is questionable, because allergens can irritate the airways by direct enzymatic action, which is not mediated by a T-cell mediated (atopic) mechanism, and will not be identified by of measuring IgE or performing SPTs.

Indeed, it is clear that the complexity of the immunological basis of asthma and allergy vastly outstrips the current scientific knowledge, and therefore it is vital to test all hypotheses.

#### *Other causal factors / effect modifiers of asthma*

Asthma is a multifaceted disease, and there are a wide range of factors that are involved in its causation and which modify its severity. Included amongst these, are psychological factors, which act via neural interconnections between the brain and the immune system [11]. The potential confounding role of psychological processes must be measured and taken into account in research.

### *Why asthma is important*

The prevalence of asthma in New Zealand is one of the highest in the world, with as many as one third of children reporting asthma symptoms among the Māori population and 8.3% of adults having doctor diagnosed asthma [12, 13]. Childhood asthma places a significant burden on children, their families, and the community. Asthma affects not only the respiratory health of asthmatic children, but also their general health and quality of life through reduced physical activity, days off school, and reduced opportunity to interact with peers and learn social skills. Asthma has downstream effects on children's families, and results in reduced income and loss of productivity when parents take time off work (paid and unpaid work within and outside the home) [14, 15]. The community as a whole pays a price for childhood asthma in terms of lost productive work from the economy as parents must look after sick children, and also increased healthcare and pharmacotherapy costs. Asthma medications are very expensive, and economic considerations can limit access to more effective newer medications because they are much more costly [16-18].

### *Why allergen reduction is a relevant topic for investigation, and what are the flaws and where are the gaps in current knowledge?*

The evidence that asthma control may be improved by allergen-reduction interventions is not conclusive. It is necessary to have good quality evidence, with a high degree of certitude in order for allergen-reduction to be taken up and adopted by healthcare providers [19] and urban planners [20]. Indoor allergens have been shown to cause

worsened asthma in asthmatic children who are atopic and sensitized to airborne inhalable allergens such as, house dust mite (HDM) allergens, viruses, passive smoking, mould, cats, dogs, birds, cockroaches, and rodents. HDM allergens play a particularly prominent role in asthma, with sensitivity to mite allergens being associated with the development of asthma [21-23], children exposed to more than 10 µg of HDM per gram of dust have more hospital admissions for asthma [24-26], and children who have a late reaction to inhaled mite allergen experience more severe asthma [27]. Allergen challenge increases levels of eNO, a marker of inflammation and asthma severity, which suggests that a decrease in allergen levels may well reduce eNO levels and improve asthma [28]. Indeed, a small study in the Italian alps demonstrated improved eNO after three months in a low allergen environment [29].

Medical knowledge of the biological mechanisms underlying asthma, suggests that the removal of the offending allergens, should result in improved asthma. This hypothesis was supported by early observations from the 1920s to the 1990s, that taking asthmatic children to low-allergen environments, such as the European Alps, improves markers of asthmatic severity [23-35]. The hypothesis that allergen-reduction would improve asthma is also supported by the fact that seasonal variation in HDM allergen loads is correlated with the seasonal variation in asthma exacerbations [25]. Moreover, exposure to lower levels of HDM allergen has been associated with a lower risk of developing sensitivity to HDM allergen [30].

The observations outlined above, led to early clinical trials of allergen-avoidance from the 1920s to the 1990s, which found beneficial effects on asthma from allergen avoidance. However, the conclusions of these studies may have had undue influence on the direction of subsequent research, considering that (i) these studies enrolled only very small numbers of subjects, (ii) their conclusions were not always statistically significant, (iii) they were uncontrolled, (iv) their results were heterogeneous (some features of asthma improved others did not), and (v) some of the improvements in asthma were only temporary. For example, a study of at least two-month residence in the low allergen-environment of a hospital ward found improvements in bronchoprovocation (increased PD<sub>30</sub> dose) and reduced dose of asthma medication. But although these results were statistically significant, the sample size was only nine subjects, they were not randomly selected from the asthmatic population and hence may be unrepresentative of atopic asthmatics in general. Because the subjects had had recent severe exacerbations of their asthma (five had been admitted with severe bronchospasm and four had been referred to specialist advice), the natural history of asthma suggests that their asthma was likely to improve spontaneously and hence their improvement cannot be clearly attributed to the low-allergen environment [31]. If there had been a control group in this study, it would have been possible to look at this group's change in asthma outcome to see if subjects recruited from that population could have improved as part of the natural history of their condition. In a controlled trial by Murray and colleagues, allergen-impermeable encasings and cleaning of bedrooms to hospital standards, resulted in improved peak flow, PD<sub>20</sub> dose, medication use, and symptoms. However, the subject allocation was not randomised, the duration was only four weeks, only twenty children were enrolled and

the control group received no placebo intervention, hence the improvement may have been due to the placebo effect [32]. Another early study, which only enrolled 14 children found improved bronchoprotection and reduced medication use after an eight-month stay at high altitude in the Italian Alps, but this study did not use a control group [33].

Subsequent trials, which aimed to test these early promising findings, with more rigorous methodologies (such as randomised controlled intervention trials, sub-group analysis by type of asthma and allergen-sensitivity status, and enrolling enough subjects to adequately power the study), have produced much less optimistic results, with some finding a benefit of allergen reduction, and some finding no benefit. Indeed, an observational study, which measured exhaled nitric oxide before and after eight months in the Bavarian Alps, enrolled a large number of children (187 boys and 124 girls), compared intrinsic with extrinsic asthmatics, and HDM sensitive with HDM insensitive subjects, which allowed them to show that although subjects' eNO improved significantly at the end of eight months compared to the beginning, the improvement was no bigger in those who were atopic compared to those who were not [34]. Hence this study gives good evidence that allergen-reduction cannot have improved these children's asthma by an immunological antigen-recognition mechanism, but instead it must have been the result of a non-atopic mechanism, such as reduced irritation from antigens mediated by a direct (non-immune) proteolytic irritation of the airways by Derp1, or by non-proteolytic innate immunologic mechanisms (such as, activation of Lipid Binding Sites and Toll-like receptors) [35-51]. Alternatively, the improvements in asthma may have been caused by some other factor about a holiday at high altitude (such as, inter alia, improved asthma

management, less stressful environment, reduced air pollution, increased ultraviolet light exposure). These other factors are more likely to have caused the improvements in asthma, because they explain why people with non-atopic intrinsic asthma got as much of an improvement in their asthma from being at high altitude as atopic extrinsic asthmatics [52]. However, no matter what the mechanism by which allergen-reduction might improve asthma, few trials produced strong consistent evidence that objective asthma outcomes improved from lowered allergen levels.

Some randomised controlled trials have found benefit from allergen-reduction interventions, but their conclusions are of dubious value because, although they employed more sound study designs than the earlier trials, they had extremely small sample sizes and examined many different outcome measures, which reduce the power of studies to find statistically significant results. When these studies were replicated using larger sample sizes, the null hypothesis was not rejected. For example, a randomised, double-blind, crossover study of only 13 HDM sensitive asthmatic children [53], demonstrated that removal of allergen from the air using a laminar airflow hood over the child's head, resulted in a reduction in the required dose of some asthma medications, but no change in symptom-free days or symptom severity. This study used multiple comparisons, by assessing the effect of the laminar airflow device on many different asthma outcome measures and by analysing each outcome measure in a number of ways. For example, changes in asthma medication were analysed for each medication individually, which found no change, but when changes in all medications were summed together, a  $p < 0.05$  reduction was found. However, this would not have been considered significant if the

authors had adjusted for multiple comparisons. The frailty of this trial's conclusions was exposed when air filtration was re-examined in studies that had a larger sample size, and that used a randomised controlled design; for example, no reductions in medication or symptoms scores were observed in a study of 32 subjects [54], or in a study of 35 subjects [55]. Trials that only enrol small samples, and which make multiple comparisons, run the risk of incorrectly rejecting the null hypothesis, particularly if they have other weaknesses in their study design. Studies that utilised randomised placebo-controlled designs and enrolled large samples have not found significant durable objective improvements in asthma [56, 57].

The abundance of methodological flaws in studies of allergen-reduction is the most likely explanation for the lack of agreement between the results of these trials. If future studies use similar methodologies, and replicate these same flaws, they will not be able to cast any further light on the subject, or reliably adjudicate on whether the allergen-reduction hypothesis should be accepted or rejected. The role of methodological flaws was raised in the meta-analysis by Gøtzsche and colleagues, who noted that:

1. some trials that reported no improvement in asthma did not actually reduce allergens and therefore could not be expected to improve asthma;
2. the design of many trials did not encourage high levels of compliance with the conduct of the interventions by subjects;
3. in addition to being methodologically flawed, many trials had extremely small sample sizes;
4. there is evidence of publication bias in favour of small flawed studies.

This issue of methodological flaws was also a feature of the systematic review conducted as part of the present MPH thesis (see Section 6.7 below).

Not only are there methodological challenges for allergen-reduction research to overcome in terms of theoretical issues (such as sample size, randomisation and blinding), there are also methodological challenges in terms of practical difficulties. For example, the natural decay of HDM allergen in the environment is extremely slow (half-life of approximately ten years) and hence pre-existing allergens need to be removed, and then the re-accumulation of new allergens needs to be measured, otherwise the massive historic allergen load present at baseline will still be present at the end of the study and it will not be possible to tell if the interventions killed the HDMs and reduced the production of new allergens [58]. In addition, it is necessary to enrol subjects whose lungs have the capacity to improve (demonstrated by improvements in PEFV after inhaling a beta-agonist), because chronic asthma leads to long-term irreversible airway remodelling and irreversible obstruction [59-63].

Despite the many flawed trials, it is clear that research in this area should continue, and is continuing, because there are compelling arguments that support the allergen-reduction hypothesis, based on medical knowledge of immunology and allergy, and several small positive trials. For example, Harving and colleagues conducted a randomised controlled trial in 30 asthmatics which produced statistically and clinically significant reductions in HDM allergens, which were correlated with improved symptoms, in asthmatic children who were moved to new homes with mechanical ventilation, compared to children who

remained in their current homes [64, 65]. In addition Morgan and colleagues found that a multi-faceted intervention did indeed improve asthma symptoms and reduce healthcare utilisation [66]. However, both these encouraging trials had limitations, because the improvement in both studies may have been caused by psychological effects rather than allergen-reduction, since there was no placebo intervention and neither the subjects nor researchers were blind to the treatment allocation. The necessity of placebo intervention and effective masking, to the assessment of a causal association is discussed in detail within this thesis.

It is vital that future research in this area is designed and conducted by a collaboration of a wide variety of researchers who have worked in this field, because, as was mentioned above, there is evidence of publication bias in favour of small poorly designed trials that had positive outcomes, and hence it is possible that large methodologically robust trials have been completed, but have not been published because of publication bias [67].

#### *Role of environment and community trials*

There is no point in testing whether putative allergen-reduction interventions reduce allergens and improve asthma, unless those interventions can be readily undertaken by families with asthmatic children. This requires research which has a high degree of external validity, so that the conclusions will be relevant to home environments in real-life communities. However, the studies must also have a high degree of internal validity, which requires stringent scientific methods, but it is difficult to maintain the rigor of the scientific method in children's homes. Clearly, there is a need to strike a balance

between what is possible in the community and the desire to use the scientific process to prove the concept that, at least under ideal circumstances, it is possible to improve asthma through allergen-reduction.

*Applying the scientific method in community based trials*

A number of elements of the scientific method are not readily transferable to the domestic environment. The requirement to blind researchers and subjects to their study allocation presents a particular challenge in the home environment, because it is hard to conceive of active and placebo interventions that have an identical external appearance. The placebo and active interventions must be identical in appearance, because the subjects are the ones who actually do the intervention procedures, and they live in small communities where they are likely to meet subjects who have been allocated to a different group, and they could talk about any differences in the intervention.

*The Morgan study was a sentinel study*

Prior to the Morgan and colleagues study, there was little agreement regarding the effectiveness of allergen reduction. The Morgan study conclusively demonstrated a reduction in allergens that coincided with improved asthma. This study was able to do so because it reduced a wide variety of allergens to which the children were sensitised, rather than just reducing one or two allergens. It also addressed social stressors, which play an important role in asthma severity. Furthermore, it was a multicentre study conducted in seven major American cities, so it was sufficiently powered to detect a small effect on asthma. The hypothesis of the Morgan study was that it is possible to

reduce asthma severity by (a) lowering of multiple allergens relevant to each child, (b) reducing environmental tobacco smoke, and (c) addressing relevant social stressors.

Because the Morgan study intervened to reduce a multitude of allergens, and it found a positive association with improved asthma, it is consistent with the assertion by some researchers, that the reason why many negative studies did not find this positive association, is that they did not reduce a sufficient variety of allergens to which the children were sensitised. However, there is a danger in using this rationale to discount the validity of studies, which found no effect of allergen-reduction. This rationale employs an argument that is unsound because there is no possible scenario under which it could be disproved (irrefutable argument); it is always possible to say “the study found no association because the children may have been sensitised to some unknown allergen that the study did not measure and did not provide an intervention to reduce it”. The association between allergen-reduction and improved asthma in the Morgan study may *not* be a causal association, because no placebo intervention was given to the control group and therefore it is possible that the placebo effect was the real cause of the improvement in the active group’s asthma.

A systematic review of literature was conducted to assess the current knowledge about the causal association between allergen-reduction interventions and improved childhood asthma [35, 37-50]. An analysis of this review is presented, and then the results of Cochrane reviews and other published systematic reviews are described. Then the

conclusions of the present systematic review are compared to the findings of Cochrane systematic reviews.

A description of the pilot study is given, including the results of a focussed search of the literature to inform the modification of the Morgan study to suit NZ conditions, and the development of placebo interventions. The outcomes of the pilot are presented, and issues relating to its conduct are discussed.

This thesis concludes with a discussion of the results of pilot study in light of what was found in the literature reviews, and outlines what design features a future study of the allergen-reduction hypothesis would have to possess, in order to allow definitive conclusions to be made.

# 1 Testing causality within the community setting

Throughout human history, a great deal of thought has been expended on how to prove that a causal association exists between two factors. Logic is an integral component to any proof, and to this end logicians have produced a set of argument structures for which the truth of the premises guarantees the truth of the conclusions (sound arguments); and a set of argument structures for which there is no such guarantee (unsound arguments, or fallacies). The scientific method utilises logically sound arguments, and requires that the proof of a causal association is clearly observable, reproducible, and not the result of some unknown or overlooked factor (termed a confounding factor in epidemiology), or the result of optimistic belief rather than ‘real’ evidence (the placebo-effect) [68-71].

In complex biological systems, in which not every element is observable or controllable, and random variation occurs, the kinds of causal relationships are complex and it is not the case that an intervention will always and invariably produce the effect that current medical theory predicts. In human health especially, there are multiple causal factors, and their role is not always a ‘sufficient’ or ‘necessary’ role, but is more commonly a ‘complex role with multiple feed-back loops’. In medicine, causality is often defined broadly: “a factor is a cause of an event if its operation increases the frequency of the event” [72]. Scientists must demonstrate that their experiments were not tainted by error, bias, confounding, or the placebo-effect (see Appendices 2 and 3).

Scientists are guided by criteria developed by Sir Austin Bradford Hill as a systematic logical method by which to judge whether a statistical association belies a causal association [73]. Although the criteria were originally developed to guide causal inference in epidemiological research, the Bradford-Hill criteria have been applied to a variety of other research methodologies [68] and they are applied in the present thesis.

The criteria are:

1. strength of the association – the bigger the effect size, the more plausible it is a causal association;
2. consistency of the observed association in different trials, different subjects, different locations;
3. specificity – the hypothesised causal agent is associated with one outcome (as opposed to being associated with many diseases);
4. temporality – the putative cause must occur before the disease occurs in order for it to be causal;
5. biological gradient – a dose-response relationship exists;
6. plausibility – it must be based on a biological mechanism;
7. coherence – the hypothesised causal relationship is not at odds with established scientific knowledge;
8. experiment – experimental evidence for the association;
9. analogy – plausibility is enhanced if it can be shown to be analogous to some other well accepted causal relationship;

10. tests of significance – statistical tests of significance cannot determine whether a causal association exists, but they can give a guide to the role of chance variation in the evidence.

It is certainly biologically plausible that a reduction in exposure to allergens could improve symptoms of asthma. In vitro experiments have demonstrated that exposure of the immune system to HDM allergens, directs the immune system toward a more hypersensitive allergic (type 2) response pattern [37]; (as was mentioned in the Introduction, type 2 lymphocytes are important in the development of asthma [6]). Derp1 is an enzyme found in HDM faeces, and it can cleave a subunit of T cell IL-2 receptors, which reduces the T cell's proliferation and IFN-gamma so that it develops into the Th2 subset [37]. Derp1 also acts directly on dendritic cells to cleave their CD40 and IFN-gamma receptor which suppresses their production of IL-12, which in turn favours the Th2 response pattern. In vivo experiments have established the allergen thresholds that cause bronchoprovocation, and therefore biological plausibility demands that interventions must reduce allergen levels to below these thresholds (or at least reduce them ten-fold) for the interventions to improve health [74]. The allergen-reduction hypothesis certainly meets the criterion of 'biological plausibility' but it is not so straightforward to judge whether it meets the other nine Bradford-Hill criteria. In particular, it does not meet the 'consistency' criterion because the studies do not consistently produce congruent results (see Table 3).

Error is avoided by the use of accurate instruments, which in community studies are often questionnaires, and other techniques to measure subjective outcomes, but error is still possible because questionnaires cannot be calibrated or objectively verified in the same way that scientific laboratory equipment can. Likewise bias is harder to identify, counteract, or avoid, in the community environment, than at the laboratory bench. Confounding is another challenge to scientists working within the community. One of the ways to minimise the effect of confounding is to randomly assign subjects to an active group and a control group. The control group serves as a comparator and should be identical to the active group at baseline. The active group is exposed to the suspected causal factor, and the control group are either not exposed or are exposed to a lesser degree. If the sample size is large enough, random allocation should ensure that potential confounding factors are equally distributed between the active and control groups, and hence cannot be mistaken for causal factors. The importance of error, bias, confounding, and control groups [72] are discussed throughout this thesis. Appendices 2 and 3 provide a more in-depth discussion.

The clearest-cut study design for proving causality, guarantees that the exposure is under the control of the scientist rather than a natural occurrence (intervention study vs. observational study), because this allows the scientist to know for sure who was exposed, and by how much, and at what time-point. It also ensures that the assignment (of who was exposed) was not the result of some pre-existing feature of the subjects, and no prior fact influenced who was assigned to the active versus control groups. This is achieved through establishing separate active and control groups, and maintaining strict adherence

to the procedures that randomly assign subjects to their allocation. These requirements are not easily maintained outside the ideal laboratory environment.

Within the community, and particularly within people's homes, it is not possible for the scientist to have full control over conditions of exposure. This is particularly so for exposures which require the subjects to behave in a certain way, such as carry out allergen-avoidance tasks. Much thought has been devoted to the development of optimal ways to modify the scientific method to make it applicable to real-world research conducted outside of the laboratory setting, while still retaining its ability to deliver rigorous trustworthy answers. Just as the family home presents challenges as a location for conducting research, so too the school-classroom causes challenges which jeopardise the conduct of scientific experiments on teaching methods, and hence it was this educational setting that formed the basis for the elucidation of experimental and quasi-experimental designs for research [75]. Randomised controlled trials can be applied in the social science setting, to prove causality, such as providing vouchers for better housing to improve asthma [76]. (See Appendix 2 for a fuller discussion).

The home is a privileged place, in which people are the master/mistress of their own wills, and it is also a private place that reflects their personality and values. To ask people to change the way they behave in their own homes is to risk breaking social conventions of polite behaviour. In particular, it is important to not offend subjects by unwittingly implying that the trial is providing them with a new cleaning regime because there was something lacking in their usual method of cleaning. The need for great tact, in

explaining allergen-reduction interventions has been noted by researchers as far back as the 1970s [77]. The home is also privileged because it is the locus of family life, which is very busy, and complicated by the substantial differences in each member's role, responsibility, capacities, motivations, and agendas. Family life is a complex unit of production, which depends on routines to ensure a regular and steady output [14, 78]. Any change in these routines, is likely to disrupt the production of a wide variety of family processes, and have a potentially detrimental impact on family life and health. This must be taken very seriously by researchers at the study design stage, because allergen-reduction studies require families to alter many of their routines. Allergen reduction studies need to take account of the routines in the ecological context of asthma, and also each family's beliefs about the causation of asthma [78].

Community trials have been conducted for several decades now, and this track-record has been examined in several systematic reviews. Community trials are research that is conducted by community workers (who typically do not have a university education) in the environment where people live, work, and play. Merzel and D'Afflitti systematically reviewed 32 community trials of disease prevention programmes, and concluded that they had only a modest impact, except for HIV programmes which were very effective (possibly due to strong HIV-community engagement, and highly effective therapies). Reasons for the poor performance of community trials include [79]:

1. methodological challenges to study design;
2. methodological difficulties with evaluation of the outcomes;
3. the overwhelming effect of concurrent secular trends;

4. smaller-than-expected effect sizes;
5. limitations of the interventions;
6. limitations of theories used.

The features of community trials that produced useful results were enumerated by Kuller's response to the systematic review by Merzel and D'Afflitti [80]:

1. a strong public health and preventive medicine science base;
2. selection of an appropriate population (e.g. a high risk sub-group);
3. use of proven intervention;
4. sufficient funding to pay for enough staff, well trained staff, and sufficient effective interventions;
5. community support.

These points raised by Merzel & D'Afflitti and Kuller are especially germane to the present thesis. Allergen-reduction trials encounter a number of methodological challenges, the interventions are of limited efficacy, and their efficacy has not always been proven even under ideal laboratory conditions. Yet, it is essential that allergen-reduction interventions be submitted to the same standards for evidence-based medicine as are other medical interventions, however when this is done it is likely to lead to the rejection of the allergen-reduction hypothesis [81].

When large allergen-reduction trials were adapted to American inner-city home environments, there was less than ideal compliance with follow-up data collection. As many as 14% of eligible children did not complete baseline measurements, and 33% did

not attend 12-month follow-up visits despite financial incentives in the large National Cooperative Inner-City Asthma Study [82]. The elements of the design and conduct of the Inner-City Asthma Intervention (ICAI) study, which were associated with higher compliance were: location of study within primary care, timely provision of asthma action-plan by doctors, onsite availability of skin prick testing, language and ethnicity of staff, and flexibility in booking appointments [83]. The fact that only a quarter of subjects fully completed this study, even though it involved substantial contact time between subjects and the social workers who conducted the trial, only underscores the huge difficulties of conducting trials in the community.

In view of the plethora of allergen-reduction trials of dubious quality, and the barriers to the conduct of an ideal study that could settle the question of whether allergen-reduction improves asthma, Platts-Mills and a team of eminent researchers have suggested a list of key components which researchers would have to take account of in order to genuinely assess the effectiveness of allergen-reduction interventions [26]:

1. not all the answers can come from a single study, so multiple studies are required;
2. need to clearly establish secular trends and natural history of atopic asthma;
3. need to enrol subjects who are sensitised to the particular allergen under investigation, and exclude those who are not;
4. long enough duration of exposure to the intervention in order for it to be able to activate the biological mechanisms upon which the intervention acts, so that it can produce a therapeutic effect on symptoms;

5. long enough study duration to allow time for a lead-in period of observation to accurately characterise baseline symptoms, asthma severity, and stabilise medication; and a long enough duration of follow-up observation after the intervention to check how durable any improvements in asthma are;
6. a control group, random allocation, and a placebo that allows for double-blinding;
7. repeated assessment of symptoms, allergen-load, lung-function including airway reactivity.

Appendices 2 and 3 discuss in more detail, the study design methods, to minimise inaccuracy due to factors such as bias, confounding, and the placebo effect.

This section has described the key methodological features that trials need to have, in order to adequately test whether there is a causal association between allergen-reduction interventions in the home, and improvements in children's asthma; the most important of which are: a randomised placebo-controlled design, accurate measurement of variables, and sufficient duration of interventions and follow-up.

## **2 Systematic literature review**

The objective of the present systematic review was to assess what is currently known about reducing allergens for the secondary prevention of childhood asthma. Its focus was allergen-reduction intervention trials directed against multiple allergens, for the secondary prevention of asthma in children aged seven to 14 years old. Children below the age of seven were not included in this review because the diagnosis of asthma does not have a high specificity in that very young age range. A summary of the studies identified by the systematic literature review is contained in Table 2. Due to the heterogeneity of the study designs, it is not possible to perform a meta-analysis, and hence the results are presented in a narrative form in which the information from the literature is organised into topics which are pertinent to the hypothesis of this thesis. Other reviewers have also used a narrative format for their systematic reviews due to heterogeneity in trial design [84, 85], although some reviewers were able to perform a meta-analysis and calculate pooled odds ratios by excluding small poorly designed studies in order to limit heterogeneity [86, 87].

It is vital that a review of the literature is undertaken in a strictly systematic manner, because the literature is vast, and studies have conflicting results. It is possible to cherry-pick studies that support the hypothesis that allergen-reduction interventions improve asthma, just as it is also possible to selectively choose studies which support the counter

argument. It was for this reason that the selection of studies for inclusion in the present review was done in a systematic way.

First, the systematic search strategy is described, and a narrative review of the results is given. Then the published systematic reviews are outlined, and to illustrate the potential for citation bias a brief mention is made of a few pertinent non-systematic topic reviews which do cherry-pick the literature [67].

## **2.1 The search strategy**

The Medline database, from 1950 to the present, was searched on 18 November 2008 using the following strategy:

(A) the MeSH subject heading “Asthma” was exploded, then the subheadings “Prevention & Control” and “Therapeutic Use” and “Therapy” were selected, resulting in 15,055 articles;

(B) the MeSH subject heading “Allergens” was exploded, resulting in 25,944 articles;

(C) A and B were combined with ‘AND’ and then limited to “Human” resulting in 1,187 articles;

(D) both the MeSH subject heading “Child” as well as “Adolescent” were searched and combined with ‘OR’; this combination was then limited by the age range because “Child” includes six year olds, and “Adolescent” includes 15 to 18 years olds, which produced 1,841,138 articles;

(E) C and D were combined with ‘AND’ resulting in 539 articles.

After reading the abstracts of 539 articles, 27 were found to meet the inclusion criteria (investigated allergen-reduction interventions in children aged six to 18 years old with asthma). The 510 articles that did not meet the criteria failed to do so for a range of reasons: either they were reviews, meta-analyses, opinion pieces, letters discussing other articles, tested primary prevention or hyposensitisation [88], or were about adults only. A summary of each of the 27 included studies can be found in Tables 2 and 3.

## **2.2 Range of study designs**

See Table 2 for detailed information about the study designs.

### **2.2.1 Randomised Controlled Trials**

Nineteen randomised controlled trials were identified [56, 66, 89-102].

### **2.2.2 Placebo-controlled RCTs**

Fifteen studies had at least a partial placebo for the control comparison [56, 89-92, 94-96, 100-106].

Few studies provided the control group with a full placebo equivalent that was identical in appearance to the active intervention. This is because there are a number of technical and practical difficulties in deploying a placebo intervention that meets the conventional criteria for an ideal placebo. These issues are discussed in detail in the section “Methods for Placebo” below.

### **2.2.3 Three-way RCTs (Factorial Design)**

Four studies attempted to separate out the specific contribution, that each of the different components of the interventions, made to the overall outcome, by using a three-way factorial design, which requires a very large sample size to ensure adequate statistical power [91, 94, 101, 104]. Ideally a four-way factorial design should be used, but these researchers used a three parallel group design to save money and simplify the trials. For example, in addition to comparing the active and placebo interventions, Carter and colleagues [91] examined the effect of having a non-placebo controlled group as well.

The study by Ehnert and colleagues [94] consisted of three parallel groups, one which received active benzyl benzoate foam and powder for mattresses and carpets, one group received a placebo version of these interventions (placebo foam and powder), and the third group was given bedding encasings and tannic acid carpet spray. However the interventions given to these three groups do not allow the role of the different components to be compared between all three groups, since it used two different acaricides.

McConnell et al [101] also attempted to tease out the effect, of professional cleaning from that of cockroach bait, on cockroach allergen levels [101], by using just three parallel groups:

- A. professional cleaning with insecticide bait traps;
- B. professional cleaning with placebo (non-insecticidal) bait traps;

C. no cleaning or bait traps.

There was no group that received insecticidal bait traps with no professional cleaning.

A trial of air cleaners with and without allergen-impermeable mattress encasings, used a factorial design [104], however it had only three arms rather than four (there was no placebo encasing), and therefore did not assess the combination of placebo air cleaner with placebo mattress encasing.

#### **2.2.4 Four-way factorial design**

Warner et al [107] tested the effect on HDM allergen levels and asthma symptoms, of mechanical air ventilation and HEPA filter vacuum cleaners in combination, and separately, compared to no intervention. The Morgan article identified in the systematic search, was actually one component of a two-by-two factorial design which looked at physician feedback as well as the allergen-reduction interventions that were described by Morgan and colleagues [66]. The advantage of this design is that it can be an efficient way to test two potential causal factors at once, however, unless it can be demonstrated that there was no statistical interaction between the two causal factors, it exposes the trial to the risk of making a Type One error due to multiple comparisons.

#### **2.2.5 Randomised placebo-controlled cross-over design**

A trial of air ionisers and bedding encasings utilised a randomised controlled cross-over design with an active and placebo periods [106].

### **2.2.6 Non-randomised controlled trials**

A Japanese study employed a controlled trial design, but did not randomise the subject allocation [108]. Likewise, a British study did not randomly assign subjects between the active or control groups [109].

### **2.2.7 Non-controlled, non-randomised, before-and-after studies**

Boner et al undertook a before-and-after trial of taking children to live for a short time in the European Alps, and because there was no control group, it is not possible to know whether the improvement in children's asthma was due to the reduced exposure to mite allergens during the stay at high altitude, or if the improvement was caused by the reduction in stress from the fun holiday in the mountains [110].

A German group also used a non-controlled before-and-after comparison to assess the effectiveness of a heater to remove allergens (such as HDM and cat) from carpet by creating convection currents to lift the allergens off the carpet, and then adsorb them onto the heater [111].

### **2.2.8 Blinding**

It is necessary to randomise subjects between an active and a control group, but a major barrier to having a control group, is the ability to blind (mask) subjects (and researchers) to their treatment assignment. Blinding is important to remove the impact of

‘psychological expectancies’ from the outcome, so that only physical processes are left to operate. A fuller discussion of blinding is provided in the section below “Methods of Blinding”. More detail about the studies that are mentioned in this section can be found in Tables 2 and 3.

#### *Open-label studies*

The majority of studies were open-label [95, 96, 107-111]. Six studies were reported as being single-blind, but the interventions in two of the groups were not identical in appearance, and hence subjects would have been unmasked if subjects from one group talked to those in the other group(s) [93, 94, 97, 98, 101, 112].

#### *Open-label but blind collection of outcome data*

The study by Morgan and colleagues could not mask the subjects or the staff who provided the interventions, but claimed to have masked the staff who collected the outcome data by using separate staff, who collected the data by telephone [66].

#### *Single-blind studies*

As mentioned above, some trials would have been single-blind if subjects in different groups did not communicate with each other, but if they did, then their masking would be ineffective and to all intents and purposes, they would be open-label [94, 97, 98, 101, 112]. The researchers were not masked in the trial by Carter and colleagues, but although the subjects in the non-placebo-controlled group would not have been blind either, it is possible that the subjects in the other two groups were blind to whether they were in the

active or placebo-controlled groups [91]. The trial by Marks and colleagues was more likely to be single blind than open-label, because subjects in both groups received either an active or placebo acaricide, however the control group did not get a placebo version of the bedding encasing that the active group received, so the subjects may have been unmasked if they spoke to subjects in the other group [98].

#### *Double-blind studies*

There were nine trials that were reported to be double-blind [56, 89, 90, 92, 102-106].

### **2.2.9 Design features to assess potential confounding factors**

Only a few studies collected data on potential confounding factors. For instance, Carswell and colleagues measured a number of potential confounding factors: compliance with regular use of corticosteroid inhalers, use of gas appliances (which produce nitrogen dioxide), smoking status of the parents, cat ownership and the social class of parents [89]. Rijssenbeek-Nouwens and colleagues assessed pollen-sensitivity at baseline, and ensured that data were collected outside the pollen season for subjects who were pollen sensitive, and only enrolled subjects who had non-carpeted bedrooms [56].

### **2.2.10 Summary**

This section has shown that a wide variety of study designs were used, some of which were less than ideal, which belies the difficulty of conducting scientifically robust

research in the domestic context, such as ensuring adequate blinding of subjects and researchers.

### ***2.3 Range of allergens that were targeted and Interventions that were tested***

The studies identified in the present systematic review tested interventions that aimed to reduce a wide variety of allergens (cat, dog, rodents, cockroach, HDM, mould, environmental tobacco smoke) which are outlined in detail in Table 2. The interventions that were assessed included: mattress encasings, hot water laundry, acaricides, education, repairs of the structure of the house, air filtration, and a holiday at high altitude.

### ***2.4 Outcome measures***

There are a plethora of outcome measures for asthma and allergen-reduction, which can lead to several scenarios in which the null hypothesis would be rejected incorrectly. Firstly, researchers could measure many different potential outcome measures, and only report the ones that were statistically significant, without mentioning the ones that were not significant. Secondly, researchers could report the positive and negative findings, but not adjust the test for significance to take account of multiple comparisons. These situations can be avoided by:

1. determining the primary outcome and analysis plan a priori;

2. publishing this plan in a trials register before any of the subjects are recruited;
3. adjusting for multiple comparisons by reducing the limit for the p-value that will be considered to be significant.

A range of indices of change in asthma were reported (see Table 3), including FEV<sub>1</sub>, FVC, FVC/ FEV<sub>1</sub>, PEFR (morning PEFR, daily PEFR, diurnal PERF variability), eNO, provocative dose of methacholine or histamine that caused a 20% reduction in FEV<sub>1</sub>.

A variety of different methods of measuring HDM allergens were reported. The most common unit of measurement was the concentration of allergen in µg of allergen per g of dust. The absolute value of allergen was sometimes reported (µg), instead of the concentration (µg/g), which provides no indication of whether this is greater or less than the threshold for exacerbating asthma [103].

Despite the wide variety of outcome measures that were reported in each study, only one study in this review made reference to adjustment for multiple comparisons [93]. Eggleston and colleagues stated that they used generalized estimating equations for comparisons to account for multiple comparisons [93].

#### **2.4.1 Allergen-reduction as the primary outcome-measure**

Two studies only assessed the reductions in allergen levels, without measuring any clinical asthma symptom outcomes: Cockroach allergen levels [101]; HDM allergen levels and change in SPT outcome [109].

## 2.4.2 Personal exposure to allergens

The majority of studies measured the change in the quantity of allergen in carpets and mattresses, which does not necessarily represent the allergens which subjects are likely to inhale. This is an issue, because it is only biologically plausible that allergens could exacerbate asthma if the allergens are inhaled, if they are not inhaled then they cannot worsen asthma. Some studies attempted to address this by measuring airborne allergen levels, which are more likely to be an accurate measure of personal respiratory exposure.

There are at least two ways of assessing airborne allergen levels: by filtering the air, and by measuring the dust that settles onto Petri dishes. Both these techniques were used by Carswell and colleagues [89, 90]. Interestingly, this study found that the interventions that were effective in reducing airborne and bedding allergens, were associated with improved asthma, but those that were directed against carpet allergen were not associated with improved asthma [90]. If it is true that airborne and bedding allergens are a better indicator, than carpet-allergen, of the allergen-load that gets inhaled, then if studies only measure carpet and not bedding and airborne allergens, they might not be capable of detecting a correlation between allergens and asthma severity [90]. There is good justification for using mattress allergen as a proxy for the airborne allergen that subjects inhale during the night, because at least two studies have found a correlation between mattress and airborne Derp1 [90, 113]. However, some researchers believe that measurements of total surface levels of Derp1 (measured as  $\mu\text{g}$  of allergen per square metre) provides a better indication of the allergen that gets inhaled, than measurements of

the surface allergen concentration (measured as  $\mu\text{g}$  of allergen per gram of dust) [89]. Indeed, most studies report both these ways of measuring Derp1. Accurate measurement of the allergens that are hypothesised to play a biologically plausible role in exacerbations of asthma, is an essential and necessary component to any trial that seeks to test those hypotheses, yet there is no agreement on how to do this [114, 115].

### **2.4.3 Asthma outcome measures**

Asthma is a loosely defined disease, and there is no single aspect of it that can be used as a complete measure of its severity. Therefore, asthma severity is measured in numerous ways, such as: lung function (peak expiratory flow, forced expiratory volume in one second, BHR); by indices of inflammation (eNO); in terms of symptoms scores; the number of exacerbations; and in terms of its effects on life (quality of life measures, and days off school).

Two studies reported no asthma outcome measures, and only assessed the amount of allergen-reduction and/or change in SPT reactivity: [92, 101]

In an attempt to summarise the asthma outcomes of the studies, their findings have been tabulated according to each kind of parameter (Table 3).

### **2.4.3.1 Peak expiratory flow**

Measuring peak expiratory flow rate (PEFR) is an important component of asthma management plans, which give early warning of a need to increase treatment; in addition, PEFR is used to assess responsiveness to corticosteroid therapy; and changes in PEFR are predictive of a deterioration in asthma control; however they are not highly predictive of hospitalisation due to asthma and therefore it is important to also consider symptoms and the overall clinical picture [116-120].

#### ***2.4.3.1.1 Peak flow variability***

Carswell and colleagues observed no improvement in peak flow variability despite lower allergen levels in active vs. control (although control groups also had reductions in allergens vs. baseline) [89, 90, 103]. Likewise, a further six of the eight studies that assessed peak flow variability found no improvement [56, 66, 100, 104, 106, 107], and one trial did not report it despite having measured it [91].

#### ***2.4.3.1.2 Morning peak expiratory flow***

Carswell and colleagues saw similar improvements in morning peak flow for both active and control groups [89, 90, 103]. Mean daily peak expiratory flow (as a percent of predicted value) at six weeks when allergen-reduction was greatest was 99.6% (SD 17.8) and 98.9% (SD 14.5) in active and placebo groups respectively [89, 90, 103].

#### ***2.4.3.1.3 Frequency of peak expiratory flow readings below 95<sup>th</sup> confidence interval***

No significant difference in this outcome occurred despite moderate allergen-reduction in the study by Carswell and colleagues [89, 90, 103], or in the study by Reiser and colleagues [102].

#### **2.4.3.2 Bronchial reactivity to bronchoprovocative testing**

Boner and colleagues observed no change in histamine PC<sub>20</sub>-FEV<sub>1</sub>, but did observe significant improvement in eosinophilic markers [121]. Carswell and colleagues found a slightly greater reduction in the slope of the dose-response curve, and the percent of children with bronchial hyper-responsiveness at six weeks in active group compared to placebo (p=0.02) but this was not maintained by six months [89, 90, 103]. Indeed eight studies found no improvement in bronchoprovocation with allergen-reduction [56, 89, 90, 95, 100, 102, 105, 107, 110], although two trials did observe a large improvement [94, 104].

#### **2.4.3.3 Eosinophils and IgE**

Two trials observed improvements in markers of eosinophil activity [104, 110], and one trial did not [105].

#### **2.4.3.4 FEV<sub>1</sub>**

Carswell and colleagues showed FEV<sub>1</sub> increased from 102.7% (SD 5.8) of predicted at baseline to 105.0% (10.2) at 24 weeks in the active groups, whereas in the placebo group the FEV<sub>1</sub> decreased from 101.8% (11.8) to 98.6% (15.3), and the difference between the groups at 24 weeks was statistically significant ( $p < 0.05$ ), although this is unlikely to be clinically significant, and even though the mean values are statistically significantly different, the standard deviations around the means of the two groups overlap. The fact that the seven other trials (see Table 3) found no improvement in FEV<sub>1</sub>, further calls into question the clinical relevance of this result of Carswell and colleagues' trial.

#### **2.4.3.5 Self-report of asthma symptoms**

Table 3 shows that, of the twelve trials that measured self-reported symptoms scores, seven showed an improvement, while five found no change, after allergen-reduction interventions. Analysis of self-report of symptoms is prone to error from multiple comparisons (see Section 6.12.2).

#### **2.4.3.6 Medication utilisation**

The majority of trials found no improvement in medication use. Table 3 illustrates that only one trial reported unquestionable improvement in bronchodilator use [89, 90, 103], one reported questionable improvement [108], and five observed no change [56, 92, 98, 106, 112]. None of the four studies which reported corticosteroid use [89, 90, 92, 98,

103], found any improvement in corticosteroid dose, even though two trials produced moderate reductions in allergen levels [89, 90, 103, 112].

#### **2.4.3.7 Healthcare utilisation**

The eight studies that report unscheduled doctor visits, Emergency Department visits, and hospitalisations, reported conflicting results, even among trials in which allergens significantly reduced. Healthcare utilisation improved without a doubt in three [66, 91, 99] trials; with some doubt in one [96]; did not change in three trials [93, 100, 112]; and deteriorated in one (but the numbers were small) [95].

#### **2.4.3.8 Functional scores/Quality of life scores**

Morgan and colleagues witnessed significant improvements in days off school, and number of times that families had to alter their plans due to asthma exacerbations [66], which was consistent with Krieger and colleagues' observation of improved quality of life [98], but not with their finding of no improvement in days off school; nor was it consistent with Eggleston and colleagues who found no change in quality of life or physical activity [93]. Williams observed improvement in the functional sub-scale, but not the total score for asthma symptoms [112].

#### **2.4.4 Evidence of potential publication bias**

Publication bias occurs when trials with positive results are more likely to be published than those with negative results [122]. Some of the articles identified in the present systematic search strategy did not provide all the expected outcome data, or just reported the design of study proposals, but even though many years have gone by, there have been no further results published on these trials. This may mean that the trials were conducted but the results were negative and therefore they were not published. For example, despite the publication of the study design for pest control by Kinney and colleagues, nothing further has appeared in the literature [97]; and despite publication of allergen levels by McConnell in 2003 [101] they once again reported allergen levels, without any asthma outcomes in their 2005 publication of their trial [123]. Similarly, Carter et al gave subjects peak flow meters, symptom diaries, and medication diaries, but do not report these data, and focus instead on rates of acute healthcare visits, which may indicate that the allergen reduction did not improve the non-reported outcomes [91].

Not only were some potentially negative outcomes not published, some positive results were published multiple times, which can create bias. Multiple publication bias occurs when a single trial is published in more than one article, and if this is not recognised by authors of systematic reviews and meta-analyses, the results of that single trial can be counted more than once, which may bias the review/meta-analysis. For example, three articles by Carswell and colleagues were detected [90], [89], and [103] which were of the same hot laundry washing + mattress-encasing + acaricide to kill HDM experiment, which may bias a reviewer toward overestimating the effect of this intervention. The

number of times a study is reviewed can also bias readers of the literature to place undue emphasis on the results of some studies over others. For instance, not only was the Carswell, Oliver, Weeks and colleagues trial reported in three articles, it was also reviewed at least once [124].

#### **2.4.5 Summary**

Section 2.4 has shown that a broad array of asthma outcomes were measured, and that overall they tended to show no effect of allergen-reduction interventions on objective outcome measures, but the more subjective outcome measures did tend to improve. Evidence of publication bias suggests that there may have been an under-emphasis and under-reporting of negative outcomes.

### **2.5 *Methods for placebo***

A placebo is an essential component of a study, to take into account psychological effects (placebo effect, Hawthorne effect, and Pygmalion effect), and also to control for practical issues such as the possibility of mistaking dilution of allergen concentration for the denaturing of allergens in a non-placebo controlled trial of acaricides. Researchers have approached the challenge of a placebo-controlled design in a variety of ways (see Table 2).

Some trials provided a placebo version of each and every active intervention. These tended to be studies of interventions which were easy to match with an identical looking placebo equivalent. It is particularly easy to make a non-active version of acaricide foam and powder, and trials of these interventions did indeed use placebos of identical appearance to the active [92, 102, 105]. Likewise a placebo version of an air ionizer can readily be constructed [106].

Some more complex trials of multiple interventions, also gave the control group a version of each of the active interventions, however the placebo version was not always truly identical in appearance, particularly for studies in which the active group used hot water laundry or mattress encasings. Two studies provided similar looking (but not identically looking) placebo versions of each and every active intervention (hot water laundering, mattress encasings, acaricide) [89, 90, 95, 103]. A similar trial of laundering, encasings and pesticides, provided the placebo-control groups with similarly appearing control interventions [91], as did studies of encasings and laundering [56], and encasings and air-filters [104]. Similarity in the appearance of the placebo can be achieved by provision of a less intense version of what is given to the active group. For example, a study of substantial building repairs was unable to provide the control group with a placebo version of the repairs, but did give them some of the other interventions that the active group received (action plans education, problem solving skills), which may have taken account of the placebo effect as long as the subjects in the control group did not talk to those in the active group [96]. A Japanese study of intensive home-visit education also

utilised a less-intensive version of the active, by giving the control-group standard-care education, as a placebo intervention [108]

Other multi-factorial trials only had a partial placebo version; either they provided a placebo version for some (but not all) of the active interventions, or they provided a placebo version to some (but not all) of the comparator groups. The control group received the same active education as the active group, but did not receive a placebo version of the home remediation [96]. An Australian study of encasings plus acaricide used a placebo version of the acaricide but not of the encasings [100]. Likewise, a trial provided a placebo version for the insecticide bait intervention but not the professional cleaning intervention [101]. Ehnert and colleagues' acaricide sub-group had a placebo control counterpart, but not an encasing counterpart [94]. There was no placebo for the mechanical ventilation or HEPA-filter vacuum cleaners in one study by Warner and colleagues [107], and no placebo equivalent for the acaricide in their other study [109].

Three trials not only had a placebo-controlled group, but also a non-placebo-controlled group as well [91, 105, 107].

Four studies gave the control group a delayed interventions that was identical to that given to the active group [93, 97-99, 112]. One of these studies gave the control group a less intense version of the active intervention in parallel time with the intervention given to the active group and then also gave the control group the full active interventions at the end of the trial [98, 99].

### **2.5.1 Delayed**

The control group received a delayed intervention in four trials [93, 97-99, 112]. This is an elegant study design in some respects, because during the delay the control subjects will be anticipating that they will get the full active intervention, and do not realise that the delay in is being used for control observation, and hence are getting the Hawthorne Effect from being observed and the Placebo and Pygmalion effects from their anticipation that they will receive the intervention in the future. Krieger and colleagues [98, 99] provided the control group with a less intensive version of the active intervention while they were waiting for the full intervention, which would have given the control group a Hawthorne/Pygmalion/Placebo effect that better matched what the active group might have experienced, compared to delayed intervention studies that gave nothing to the control group while they waited for the full intervention. However, this has the disadvantage of potentially improving the health of the control group and obscuring any positive impact of the active intervention. The use of an identical but delayed intervention for the control group may not completely remove the placebo/Pygmalion effects because the subjects in the control group may become demoralized if they have to wait too long for the interventions, and therefore the Placebo and/or Pygmalion Effects that they may have initially got from the promise of future interventions may fade with time.

## **2.5.2 More intensive versus less intensive**

Some studies have overcome the problems of trying to invent a placebo intervention that will match the active intervention, by giving both groups identical, or very similar, interventions, but given a more intensive version to the active group and a less intensive version to the placebo group. For example, Krieger et al [98] gave both active and control groups identical bedding encasings and asthma action plans, but only gave full education to the active group and just gave limited education to the control group, and only gave the active, but not the control, group multiple home visits (seven versus one home visit), rodent and cockroach avoidance equipment, and social support. In a similar manner, Carswell et al instructed the active group to launder with hot (60°C) water but told the control group to wash with (40°C) [90].

## **2.5.3 Issues with placebos that have a mild positive effect**

One disadvantage of the more intensive versus less intensive interventions, as an alternative to an ideal non-active placebo, is that it may reduce the effect size of the study. For some allergen-reduction interventions, in order for their placebo-version to have an identical appearance it must have a mild positive effect. For example, placebo air filters entrap substantial amounts of dust and allergens, which may obscure any improvement in the active group that might have come from the dust entrapped by the active air filters [104]<sup>1</sup>. Also, placebo bedding encasings trapped substantial amounts of allergen despite supposedly being allergen-permeable, which would diminish the ability

of trials to detect any improvement in asthma from the active allergen-impermeable encasings [95] (see the heading below ‘Issues with placebo encasings’). Furthermore, there was a large reduction in allergens with Dietemann and colleagues’ placebo acaricide spray (statistically significant for guanine but not Derp1 or Derf1), which may explain why their study revealed no statistically significant difference in asthma between the two groups (the placebo spray contained tensides which may have reduced allergen load measurements, however it is more plausible that the placebo effect was the cause of the lack of difference in asthma) [92].

Although Marks and colleagues did not use a placebo mattress encasing, the HDM allergen levels reduced in the placebo group as well as the active group. Two weeks after the interventions were undertaken, the levels were 29% of baseline in the active group (a reduction of 71%) and were 61% of baseline levels in the placebo group (a reduction of 39%), after the initial two weeks there was no statistically significant difference between the allergen levels in the two groups [100]. Clearly, the fact that allergens reduce substantially in a placebo group even though they were not given a placebo encasing, suggests that just being in a trial causes a reduction in allergens (perhaps through more frequent washing of bed linen).

#### **2.5.4 Issues with placebo encasings**

Active bedding encasings are designed to act as a barrier to mites and their allergens. Active encasings rely on either very tightly woven cotton (that has only a very small gap

between the cotton threads) or a solid polyurethane layer. For the active encasings, the pore size (the gap between threads) needs to be as small as 2µm to 6µm in order to block allergens completely, so the placebo encasings need to have larger pore sizes than this, but even very low-quality loose-weave sheets, with pores more than twice as large (20µm), block 95% of Derp1 [125]. So even the flimsiest of fabrics, which could be used as a placebo, will still block the vast majority of allergens, and they will also block many of the mites themselves, because mites in their smallest life-stage (larvae) have a diameter of 20µm or more [126]. In addition to the mite and allergen-blocking abilities of placebo-encasings, the control group subjects may also wash their sheets more often than they did previously, which will also reduce allergen concentrations in their beds because mite allergens are water-soluble.

Hence, it is not surprising that three studies observed a reduction in allergens in the placebo group. In one study, there was a 3-fold fall in bedding allergen in 5/7 placebo homes, that initially had elevated levels [95], another study also saw a fall in allergens with the placebo [91]. In one study, the decrease in allergens in the placebo-encasing group was small enough that it did not obscure the statistically significant reductions that occurred with the active encasings [89, 103].

Not all placebo encasings reduced allergen levels. The placebo encasings used in the Rijssenbeek et al study did not reduce Derp1 levels significantly, whereas the active encasings did significantly reduce them [56].

It is not possible to make a placebo encasing that looks and feels identical to the active version. This is important because for a trial to be double-blind, and for the control subjects to experience a placebo-effect, the placebo encasings need to have an identical appearance to the active encasings. Although some researchers report that their placebo encasings were identical in appearance to the active ones, this is not always a plausible claim. For example, Hayden and colleagues state that their polycotton non-urethane coated placebo encasing was outwardly identical to the urethane coated active encasing [95], however the urethane coat changes the textural feel of the fabric, and the sound that is makes when it is crumpled by the body-weight of the occupant of the bed. This urethane coating makes the active encasings much less comfortable than placebo ones, which explains why there were statistically significantly more complaints about the active encasing (8 subjects) compared to the placebo encasings (0 subjects) [89]. This difference in sensory effects may unmask the subjects and/or the research staff (see section on blinding below).

Not only are there problems with placebo encasings, there are issues with active encasings too, with respect to the longevity of their allergen-reducing effect. There is no bedding encasing that has been proven, beyond doubt, to reduce allergens over a sustained period of time. Polyurethane fabrics reduce allergens in the short-term (up to twelve weeks) [127], but mites are able to infiltrate and live in little pockets within the polyurethane, and despite early reductions in allergens, they quickly re-accumulate within a few months time [128, 129].

### **2.5.5 Absence of placebo for some interventions**

Some studies did not provide placebo equivalents to match all of the active interventions. Some active interventions are very difficult to develop placebo interventions for. For example, it is difficult to imagine a placebo equivalent for professional home cleaning [101]. Marks, Tovey et al 1994 did not provide a placebo equivalent for the bedding encasings, yet they claimed the study was still single-blind, which would only be true if the subjects in the control group never met those from the active group, and if the subjects in the active group did not pass comment about the covers to researchers collecting the data, which could bias researchers (wittingly or unwittingly) to collect asthma symptom data more intensively in one group compared to the other.

In a home remediation study to remove mould, the control group received a placebo in the sense that they got similar education as the active group, but they did not get remediation of their homes [96]. Consequently neither the subjects nor the researchers were blind once the intervention period began, which is likely to have influenced the way control subjects viewed the trial since more of them were lost to follow-up than the active group (24% cf. 10%).

If a trial does not match every active intervention, with a corresponding placebo intervention, then it leaves open the possibility that the improvement in the active group's asthma was merely a placebo, Hawthorne, or Pygmalion effect.

## **2.6 Methods of blinding**

There was considerable diversity in the methods used to aid the masking of subjects and researchers (Table 2). There are numerous practical barriers to the conduct of a blind allergen-reduction trial. Most of these barriers relate to the design of placebos that look the same as the active, but which neither increase nor decrease allergen levels (see placebo section above). Some researchers were moderately successful in their blinding, others claimed their studies were single or double blind, but the veracity of their claims is doubtful.

Carswell et al [89, 90] were the only group that asked subjects at the end of the trial what group they thought they had been assigned to. The blinding in their study was moderately successful, since only 46% thought they knew which group they had been assigned to, and of these 46% only 52% guessed correctly. The reason that blinding was so successful in this study is that the control group received a placebo intervention that was very similar in appearance to what was given to the active group – the only differences that might have been noticeable were the laundry water temperature, and the absence of a polyurethane layer in the placebo mattress encasing.

Some trials were inevitably open-label, such as those that had a placebo that consisted of a delayed version of the active intervention, or for which no placebo could ever look the same as the active. The fact that the intervention for the control group was substantially delayed (given at the end of the study), makes it unlikely that Eggleston and colleagues'

trial was blind, despite their claim that it was [93]. They reported that follow-up contact was done by staff who were blind to the subjects' treatment assignments, however it is hard to imagine how a researcher could go to the home of a subject who had been enrolled for a long period of time, notice that they had not got air cleaners or encasings, and not immediately conclude that this subject was in the control group. Likewise, subjects who had to wait a long time for their intervention, might well have begun to wonder if they were in the control group. The study by Hayden et al removed carpets from the active group's bedrooms and replaced them with polished floors, but no equivalent could be given to the control group [95]. Likewise, there was no reasonable way for Hayden et al to blind that fact that the active group were washing their laundry in hot water yet the control group were washing theirs in cold water. Interestingly, these same hurdles were faced by Rijssenbeek-Nouwens et al, who attempted to surmount them by only enrolling subjects with non-carpeted bedroom floors, and requiring the placebo group to wash their sheets at the same high temperature as the active group [56]. The issues around inventing a placebo encasing that matches the sensory characteristics of active encasings have been discussed in the section above, and explains why van der Heide did not attempt to use a placebo version of the encasings that were given to the active group [104], however this came at the cost of potentially unmasking the trial, because the groups did not get identical looking interventions.

Trials in which the active group used hot water to wash laundry (60°C), but the control group used only warm water (40°C), may not have been truly double-blind [56, 89, 90]. If subjects from one group talked to those in the other group, then they might readily

discover that they were in different treatment assignments. The researchers might also have been unmasked, if subjects mentioned a feature of the intervention that they received which was unique to their group, and was not also a feature of the intervention given to the other group. For example, subjects in the active group might mention that they were surprised by the high electricity bill from using more hot water than usual, or that their laundry room got damp because of the condensation from the hot water vapour coming off the hot laundry, or that they end up running out of hot water for showers and baths because it gets used up doing the laundry.

Not only are some physical interventions difficult to blind - like removing carpets and matching active encasings - it is probably impossible to blind subjects and researchers if the interventions have an educational or counselling component. For instance the intensive versus standard allergen-reduction education trial by Nishioka et al was open-label [108].

It is not difficult to create a placebo version for some allergen-reduction interventions in which the placebo has an identical appearance to the active intervention. For instance, an acaricide spray for mattresses is a simple liquid, which can easily be copied in a non-active form, and packaged in identical containers [94, 102] and it is straightforward to make non-active air filters [104] and air ionisers [106]. Interestingly, interventions which lend themselves to the use of more rigorous tests of causality, tend to be the interventions for which there is conclusive evidence that they are ineffective (air filters and ionisers, for example).

Researchers and families were kept blind to whether the children were sensitised or not to HDM in the study by Hayden et al [95] and the study by Carter and colleagues [91] (by delaying until the end of the study, measurement of IgE in the blood taken at baseline) which meant that sensitisation could not be part of the inclusion criteria and some non-sensitised children were indeed enrolled, which is wasteful of resources because non-sensitised children cannot benefit from allergen-reduction. It is unclear what advantage this approach would have because subjects get randomised after enrolment, and as long as the randomisation process is tamper-proof then knowledge of the children's sensitivities is not going to affect which group they get assigned to.

## ***2.7 Poor Quality Study Designs***

By far the majority of studies had low quality study designs and these will be discussed in detail below. However, there were some notable exceptions that used sound designs, and reported their methods and results thoroughly, such as the trial by Carter et al [91].

### **2.7.1 Many studies were poorly designed and/or poorly reported**

The study by Sette et al [105] was of low-quality in a number of respects, despite being conducted in a double-blind randomised placebo-controlled design. In this study, children were taken to the Italian Alps for nine months where there are no HDMs, and

returned to their homes for two short periods, after their beds at home had been treated with active or placebo acaricide; their lung function and histamine PD<sub>20</sub> was calculated before and after their visits home. Not surprisingly, no difference was observed between the two groups. The design of this trial did not allow any effect of being taken away on holiday to be distinguished from the effect of the mattress acaricide spray, because it is conceivable that the beneficial effect of being in the Alps would have lingered long after they had returned to their beds at home and this may have prevented their asthma from worsening, regardless of the HDM allergen levels in their home mattresses. A further deficiency in the design, was that children were only re-exposed to their home mattresses for a total of 30 days out of the total study duration of nine months, which was mainly spent at high altitude, however 30 days may not have been long enough to set in chain the inflammatory processes necessary to precipitate asthma symptoms. The way this study was designed lacked attention to the biological mechanisms behind atopy and asthma, and therefore could not be expected to test the allergen-reduction hypothesis.

Two trials did not use a control group, yet studies that have no control group cannot provide definitive conclusions and are only useful for hypothesis generation, because it is not possible to tell if the improvement was caused by the interventions, a placebo-effect, or a natural change that would have occurred anyway. This is particularly so for trials whose main outcome measure is completely subjective, such as the improved symptom scores from air filtration in the non-controlled study by Fischer and colleagues [111]. The absence of a control group in Boner and colleagues' study makes it impossible to know if improvements in asthma were caused by allergen-reduction or the reduction in

stress due to the fun holiday in the mountains [110] (Appendix 3 explains the Hawthorne and Pygmalion Effects which may have improved the children’s asthma).

Ehnert et al used an illogical design which was poorly described in their publication, and hence it is not easy to deduce what interventions each group received. They used a three-way factorial design, but this did not allow for the different intervention components to be assessed individually, because the acaricide given to one half of the active group (the mattress encasing active group) was different to that given to the other half of the active group (the non-encasing active group), and there was no group that received a placebo-encasing and a placebo-acaricide [94]. Ideally, this trial would have used a design like this:

	<b>1. Active encasing</b>	<b>2. Placebo encasing</b>
<b>A Active benzyl benzoate + active tannic acid</b>	<b>1 + A</b>	<b>2 + A</b>
<b>B. Placebo benzyl benzoate +placebo tannic acid</b>	<b>1 + B</b>	<b>2 + B</b>

Instead it used the following design:

	<b>1. Active encasing</b>	<b>2. No encasing</b>	<b>3. Placebo encasing</b>
<b>A. Active benzyl benzoate</b>	<b>Not done</b>	<b>2 + A</b>	<b>Not done</b>
<b>B. Placebo benzyl benzoate</b>	<b>Not done</b>	<b>2 + B</b>	<b>Not done</b>
<b>C. Active tannic acid</b>	<b>1 + C</b>	<b>Not done</b>	<b>Not done</b>
<b>D. Placebo tannic acid</b>	<b>Not done</b>	<b>Not done</b>	<b>Not done</b>

There seems to be no justification for giving the encasing group tannic acid for their carpets, while giving the two non-encasing groups benzyl benzoate or placebo benzyl benzoate. The authors claim that the improved PD<sub>20</sub> in the encasing group was due to the encasings, however because the encasing group was also given tannic acid, while the non-encasing groups were given benzyl benzoate instead of tannic acid, it is not possible to refute the possibility that the improved PD<sub>20</sub> in the encasing group was actually due to the tannic acid rather than the encasings.

The study by Carswell and Weeks and colleagues was poorly reported because it was dispersed across at least three different articles [89, 90, 103]. These authors have wittingly or unwittingly been misleading in at least two regards. First, they report the allergen-reduction measures at more time-points than they report the asthma symptom outcomes (weeks 2, 6, 14, and 24 for allergen levels, compared to just weeks 2, 6, and 24 for clinical outcomes). I conjecture that this is because the reductions in allergen levels

were statistically significant at all the time-points but the improvement in asthma symptoms were not. The authors report that asthma symptoms were significantly reduced by two weeks, not significantly reduced at six weeks, but were once again statistically significant at 24 weeks. It seems implausible that the persistent allergen-reduction would have improved asthma, then had no effect, then regained its beneficial effect later on; and if it is the case (as I surmise) that there was no significant symptomatic improvement at 14 weeks, this would only have drawn more attention to the lack of symptomatic improvement at six weeks, and more openly cast doubt on the biological relevance of the significant improvements at 24 weeks<sup>ii</sup>. The second possible ‘misrepresentation’ by these researchers is their suggestion in their paper published in 1999, that the reductions in allergen “could mean dual benefits to a patient sensitive to both mite and cat”, yet the authors had completed this trial prior to 1995 [89, 90], and by the time they came to write the 1999 paper, they would have known that these reductions in allergen (which were reported in 1995 [103]) had improved only some asthma outcomes and had improved them only at some time-points not others.

The Japanese study of educating families on allergen avoidance techniques had copious design flaws - not only was it open-label and non-randomised, but its validity was further undermined by the fact that the researchers chose which group to assign subjects to after their allergen-sensitivity had been determined [108]. This could bias the study toward the null hypothesis if researchers felt sorry for the subjects with the more severe sensitivity and therefore wanted to assign them to the active arm of the study in the hope that it would be more effective. Alternatively, it could bias the study toward falsely rejecting

the null hypothesis, if researchers felt passionately about the merits of the active intervention and therefore assigned subjects to the active group, who were more likely to respond on the basis of their allergen sensitivities and other features of their asthma.

The enrolment process in the study by Williams and colleagues may have selected subjects who are not at all representative of asthmatic atopic children, and therefore the results have poor external validity, because enrolment was instigated by community health workers inviting families to take part in this study [112].

Warner and colleagues interfered with the randomisation of subjects, because subjects who had been randomised to the active group, but whose homes prevented the installation of Mechanical Ventilation and Heat Recovery (MVHR) units, were re-assigned to the control group instead [107].

Allergen levels are well known to not be normally distributed, which requires them to be reported as the median and interquartile range (or the log-transformed mean and log-transformed standard deviation), yet Kercksmar et al reported the allergen levels as mean and standard deviations, which is not meaningful. Not only that, Kercksmar et al appear to suggest that allergen levels can exist as negative numbers (see Table 3 on page 1577 of their report) [96].

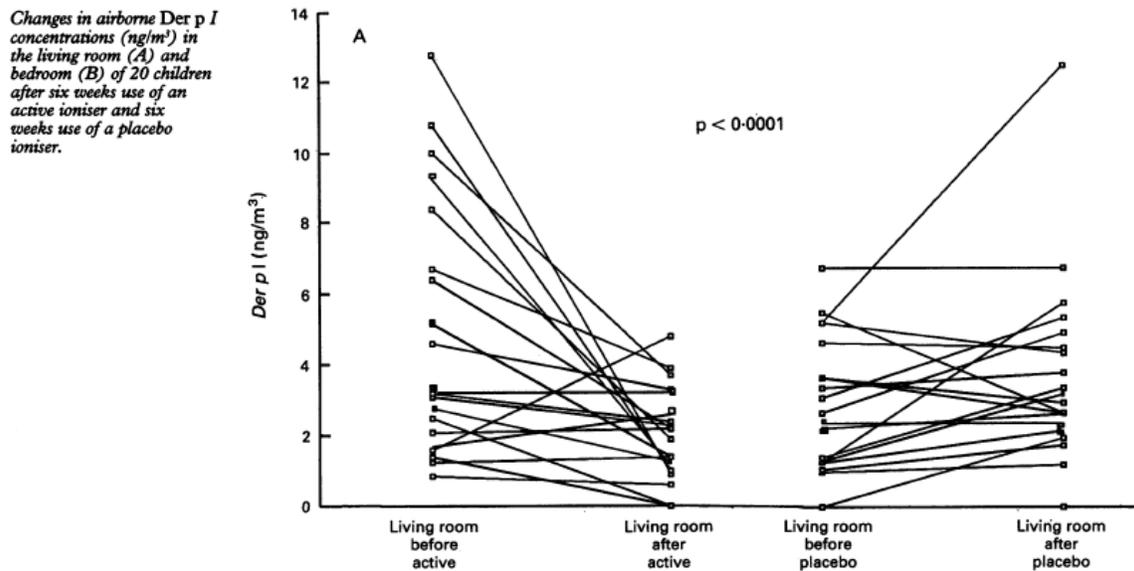
The study by Carter et al should have been able to assess the independent effect of having a researcher visit the home of the subjects, because it had a three-way parallel study design:

1. the active group got allergen-reduction interventions and home visits;
2. the full-placebo-control group got placebo allergen-reduction interventions and home visits; and
3. a minimal-placebo-control group got no allergen-reduction interventions and only minimal home visits [91].

The active group had a reduction in acute health-care visits, which was significantly different to the minimal-placebo group, but not significantly different from the full-placebo-control group. There was no significant difference in allergen-reduction in the active versus full-placebo-groups, but no dust samples were collected from the minimal-placebo control group. This failure to collect dust from the minimal-placebo group, means that it is not possible to determine if their poorer outcome was due to the fact that they did not receive as many home visits, or due to them having higher allergen levels at the end of the study compared to the other two groups. In addition, it is not possible to know if the similarity in asthma outcome between the active and full-placebo groups was due to the placebo-effect, or the fact that both had similar reductions in allergen. We can conclude that an inter-group comparison in this study only gives a confused picture, due to the lack of allergen level data in the minimal-placebo control group.

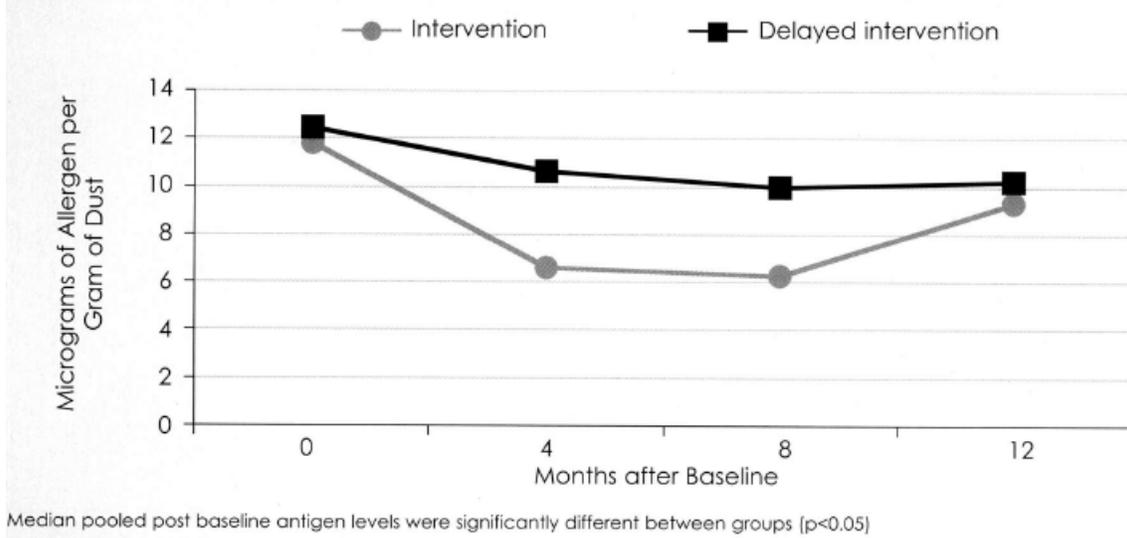
An example of a well designed, but poorly reported, study is that by Warner and colleagues [106], which, like the Sette and colleagues article, presents data solely by the

use of graphs instead of also providing summary measures such as the mean or median and some idea of the range and spread of the values. For example, although the graph that was used to report the allergen reduction shows that the difference in concentration between the two groups was statistically significant ( $p < 0.0001$ ), by missing out the mean or median difference between the two groups, it is impossible to know what the size of the difference was and whether this represents a clinically significant reduction:

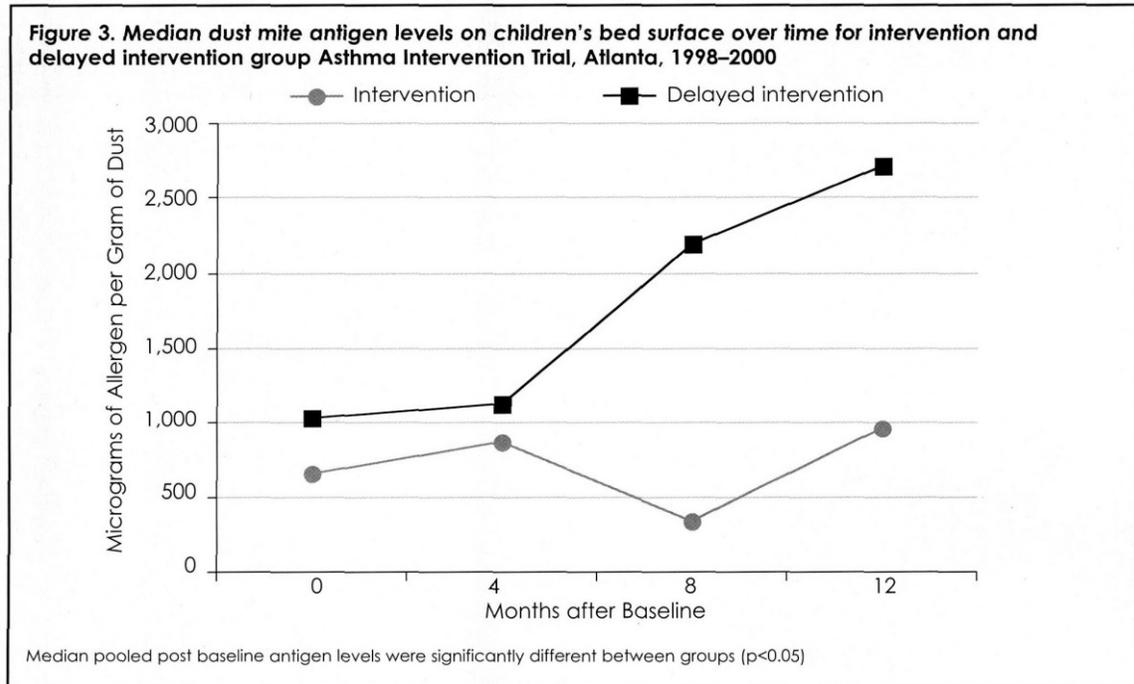


Yet another large American study used graphs to present data, but did not provide log transformed mean values (see Figure 4 below) Williams et al [112].

Figure 4. Median cockroach antigen levels on kitchen floor over time for intervention and delayed intervention group Asthma Intervention Trial, Atlanta, 1998-2000



Another example of poor reporting of results, which would appear to overstate the findings, is the sentence in the discussion by Williams et al on page 257 of their report “Dust mite allergen and cockroach allergen levels declined substantially, although the decline in the cockroach allergen levels was not sustained” [112]. While this was true of cockroach allergens (Figure 4 above); the graph reproduced shows that the HDM allergen level in the intervention group remained fairly constant, and it is misleading to state that it declined – the levels were only significantly lower in the active group than the control group at the end of the study, because allergen levels rose dramatically in the control group. The authors can only claim that the intervention prevented an increase in allergen.



### 2.7.2 Some studies had extremely small sample sizes

Many of the studies identified in this review enrolled very small sample sizes, yet small studies are much less able to produce statistically significant results. For example, the study by Boner and colleagues enrolled twelve subjects and found improvements in eosinophilic markers but not bronchial lability [110], Dietemann and colleagues only enrolled 26 subjects and found little effect of Acarosan [92], just as Warner and colleagues found no advantage with thorough cleaning and an acaricide in a study of 16 children [109] or with ionisers in a study of 20 children [106].

The randomised placebo-controlled study of combined multiple allergen-reduction interventions by Hayden et al enrolled only 23 subjects which may explain (in part) why

there was no improvement in FEV<sub>1</sub> or PD<sub>20</sub> despite the improvement in PEF, and meant that rare events like hospitalisations could not be compared in a statistically significant manner because of the small numbers (4 active and two placebo children were hospitalised) [95].

Not all studies with small samples sizes were unable to detect an effect of allergen-reduction. For example, the study by Ehnert enrolled only 24 subjects into a three-arm study, so that there were only eight subjects per arm, yet it found that the encasing group had a significant reduction in allergen and increase in their PD<sub>20</sub> [94].

## ***2.8 Cumulative dose of allergen and magnitude of reduction***

Studies varied in the size of the initial and final allergen exposure, the number of rooms in the house where the exposure was reduced, and the places within the room (mattress, carpet, settled-dust on surfaces, airborne) where allergen-reduction was demonstrated. It stands to reason, that studies are less likely to find an effect of allergen-reduction on asthma, if their allergen-reduction interventions occur in only a minority of the places where children spend time. For example, some studies only reduced allergens in the mattress, or only in the carpet in the bedroom rather than also in the living room. No studies attempted to reduce allergens in curtains, only some applied acaricide to sofas.

It is always possible to criticise allergen-reduction studies for not having a sufficiently comprehensive set of interventions and outcome measures, and this criticism has been

levelled at a number of studies, such as the criticism of the Kinney et al study [97] by Elihu Richter [130]. In order to really tackle the root cause of elevated allergen levels in homes as a whole (not just one small part of the home), then instead of only using interventions that have a very direct (but limited) mode of action in reducing allergens (such as acaricides), it is necessary to provide more generalised ecological interventions. In these broader interventions, such as remediating the structure of buildings, the causal pathway is not quite so direct between intervention and asthma outcome, however it may possible have a more pervasive and more powerful positive effect on asthma. For example, according to Richter [130] who criticised the Kinney study for providing acaricides, but not fixing the basic structural problems in slum housing in New York, the structural problems are themselves likely to cause allergens that would overwhelm any allergen-reduction effect of the interventions in the Kinney study.

It is necessary to be cognisant of the magnitude of the reduction in allergen compared to the proposed thresholds for provocation of symptoms. Some studies found little improvement in asthma from allergen-reduction interventions, because both active and control groups experienced levels of allergen that for much of the study duration were below the critical level for affecting asthma. There is debate about what is the lowest concentration of allergen that can provoke asthma, with some reporting that epidemiological evidence suggests a level of 2  $\mu\text{g/g}$  of dust is associated with primary development of sensitisation, and a level of 10  $\mu\text{g/g}$  dust is associated with worsened symptoms, after sensitisation has already developed [26]. Other researchers claim that levels as low as 2  $\mu\text{g/g}$  dust are sufficient to cause symptoms in highly sensitised people

[131]. It is important that allergen levels be high enough at baseline in order for any clinical improvement to occur after the levels are reduced. For example, a study of a multi-faceted intervention [112], reported no improvement in total asthma score, despite the HDM and cockroach allergen levels being statistically significantly lower in the active group compared to the control group; however, the HDM allergen levels were very low in both groups even at baseline, and were well below the threshold of 2  $\mu\text{g/g}$  of dust in both groups for most of the trial.

Two studies which found the interventions were effective in lowering allergen levels, only demonstrated this allergen reduction for a short duration of time, after which the allergen levels re-accumulated. For example, cockroach allergen levels reduced in both active and control groups at four and eight months, but rose at twelve months, with the rise in the active group being quite substantial [112]. Similarly, Marks and colleagues saw a significantly greater reduction in HDM allergen at two weeks in the active group, but these levels were no longer significantly reduced by three and six months [100].

In conclusion, although it is an irrefutable argument (and therefore a logical fallacy) to argue that asthma would have been improved if only allergens had been lowered more thoroughly, it is a biologically plausible argument, and therefore must be addressed. As was discussed in the preceding paragraphs, a substantial reduction in allergen requires that housing structures do not impinge on the thorough reduction of all noxious airborne substances, but conversely, it is also essential that baseline allergen levels are not so low,

that it is not feasible to lower them further. The ideal study would take these matters into account.

### **2.8.1 Intervening to reduce more than just one allergen**

It is only biologically plausible that reductions in allergens might improve asthma, if all the different kinds of allergens that a person is sensitized to are lowered. Sixteen of the twenty-three trials in the present review tested interventions against a range of different allergens, and hence this review has extracted trials that should be capable of testing the allergen-reduction hypothesis.

### **2.8.2 Synergy from multiple interventions for each allergen**

In order to achieve substantial reductions in allergen levels, it is necessary to utilise more than one intervention against each kind of allergen, because it is very hard to remove allergens. A time-saving and cost-saving way to do this is to use a range of interventions, each of which are effective for more than one kind of allergen. Levels of HDM allergens are especially difficult to lower, and not many studies have been able to reduce the concentrations of HDM allergen. In fact, it was only by restricting enrolment to children with no carpets in their bedrooms, that a Dutch study of bedding encasings was able to achieve a statistically significant (but not clinically significant) reduction in allergen [56]. However, this extreme inclusion criterion greatly reduces this study's external validity.

A number of large American studies have been predicated on the argument that a multifaceted approach is required for the secondary prevention of asthma, including the reduction of a large range of allergens, plus social and educational interventions [112].

Warner and colleagues recognised the absolute necessity of a combination of interventions in their study of MVHR [107]. MVHR may kill HDM by reducing humidity, but even if the mites are killed, their allergens will remain for many months in the environment [104], so the death of the HDM cannot be detected by measuring a change in allergen levels. Therefore Warner and colleagues gave subjects HEPA filter vacuum cleaners, in addition to MVHR, so that the pre-existing allergen levels could be removed, and any allergen levels measured at the end of the study would represent only newly deposited allergen which would indicate whether the mites had survived. The improvements in asthma in this study by Warner and colleagues may have been more pronounced if they had also used allergen-impermeable bedding encasings, because although there was a significant trend to lower Derp1 in some areas of the house with MVHR, there was no reduction on mattresses. It is the allergen on mattresses which has the greatest impact on asthma, and so this might explain why there were only non-significant trends to improved asthma in this study. Morgan and colleagues observed significant improvements in asthma and reductions in allergen from multiple interventions directed against each of a wide range of allergens [66].

The use of a combination of interventions does not always lead to an additive or synergistic effect. The combination of active filters plus encasings had a positive

synergistic effect for bronchoprovocation but a negative synergistic effect for eosinophilic markers: only the group that got both active filters and active mattress encasings, experienced improvements in their histamine PD<sub>20</sub>, whereas the groups that got just one or other of those interventions did not [104]. But eosinophilic markers only improved in the group that got active encasings and placebo filters, whereas eosinophilic markers remained unchanged in the two other groups (the group that received both active filters and active mattresses; and the group that only received active air filters). A further example of the failure of a synergistic effect of combination interventions, is provided by Carswell et al who showed that the addition of an acaricide (Acarosan foam and powder) to mattress encasings and carpets offered no further benefit in terms of mite allergen reduction [89, 90, 103].

### **2.8.3 Magnitude of allergen reduction**

In the following paragraphs in this sub-section, the allergen-concentration thresholds for provoking asthma are described, then the studies that found a positive association of allergen-reduction with improved asthma are discussed (along with their caveats), and then the notable exceptions are outlined along with possible reasons why they may have failed to detect an association. It is biologically plausible to expect to see a dose-response relationship, so that the greater the magnitude of reduction in allergen concentrations, the greater the potential for improvements in asthma. It is only possible to produce a large reduction in allergen levels if the levels are high to begin with (at baseline), yet a number of studies had fairly low allergen levels at baseline, for instance

the mean baseline Derp1 ( $\mu\text{g/g}$  of dust) in a Dutch study was only 0.97 [56]. (Studies that formally tested for a dose-response relationship are discussed in a separate section below).

The threshold for sensitisation to Derp1 is  $2\mu\text{g/g}$  of dust, and the threshold for provocation of asthma symptoms is thought to be  $10\mu\text{g/g}$  of dust [26]. Exposure to  $2\mu\text{g}$  of group I mite allergen per gram of dust (100 mites per gram or 0.6 mg of guanine per gram) is considered to increase the risk of allergen-sensitisation and asthma, and exposure to  $10\mu\text{g}$  of group I mite allergen per gram of dust (500 mites per gram) increases the risk of acute attacks of asthma [26]. However, there is evidence that some asthmatics can be provoked by allergen doses that are lower than these thresholds [26].

Carter and colleagues performed a sub-analysis of subjects who were sensitized to HDM allergens and whose allergens reduced by 70% or more. This sub-group had clinically significant improvements in asthma (reduction in acute healthcare utilisation) compared to subjects whose allergens did not fall by at least 70% [91]. However, the findings of Carter and colleagues may have been caused by the placebo effect (see the Placebo section).

Subjects in the mattress encasing component of the study by Ehnert and colleagues, experienced a large (98%) reduction in HDM allergen, which was statistically significant, and may explain why they had a significant improvement in bronchial hyper-reactivity,

while subjects in the other two groups whose allergens did not reduce did not improve their hyper-reactivity [94].

Nishioka and colleagues observed that intensive home-visit allergen-avoidance education for HDMs versus brief clinic-visit allergen-avoidance education produced reductions in mite allergen of 20.1 and 10.3 in atopic and non-atopic children, respectively, in the active group; versus 0.9 and 2.2 in atopic and non-atopic children, respectively, in the control group [108]. In this study the greater allergen reduction in the subjects in the active group was accompanied by significantly fewer asthma attacks in the active group compared to the control group [108]. However, this study is difficult to interpret for a number of reasons. First, not only did the atopic children's asthma do better with the active compared to placebo interventions, but the non-atopic children's asthma improved to. This suggests that the improvements in asthma were not mediated through an immunological pathway. Second, the authors did not provide statistical test of the correlation between the magnitude of allergen-reduction and reductions in asthma attacks. Third, it is possible that the reduction in allergen levels were a confounding factor of the association between education and asthma control - it is possible that the improved asthma was caused by the Pygmalion / Hawthorne effects because the active groups received more in-depth education (and therefore more contact time with researches) than the control groups.

Hitherto in this section, studies have had results that were consistent with the allergen-reduction hypothesis, but now studies that did not clearly support the hypothesis are described, and the reasons they were not conclusively positive are discussed.

A study by Rijssenbeek-Nouwens and colleagues produced a statistically significant reduction in allergen, but did not find any improvement in asthma, possible because even the baseline levels were well below the threshold for asthma provocation (10 µg/g of dust) [56], so even though the reduction was statistically significant, it was not biologically significant: Derp1 (µg/g of dust) reduced from a mean of 0.97 at baseline to 0.03 after one year in the active group, and from 0.73 to 0.61 in the placebo group. Likewise, there was no capacity for a large-magnitude allergen reduction in the study of Allersearch DMS by Warner and colleagues, because the baseline allergen levels were extremely low, and remained below 3.5µg/g throughout the duration of the study. Even though there were statistically significant reductions in allergen levels, and SPT reactions, in this trial, it does not provide convincing support for the allergen-reduction hypothesis because the allergen levels were so far below the provocative dose, and because the reduction in allergen may have been an artefact produced by the potential for tannic acid to interfere with the ELISA test used to measure the Derp1 levels [109].

The study by Carswell et al produced substantial and statistically significant reductions in allergen levels, including the biologically relevant aeroallergens, however this did not lead to a convincing improvement in asthma (only FEV<sub>1</sub>, bronchodilator usage, and symptoms improved, but not bronchial hyper-responsiveness, nor thrice-daily PEFR

measurements) [89, 90, 103]. This extremely modest change in asthma occurred despite a number of elements of the study design that should have ensured that the study would have been capable of detecting an association if there was one. In order to increase the potential magnitude of the allergen-reduction, these researchers only enrolled children who had the highest levels of mite allergen in their homes (as determined from a survey of allergen levels). There was a large magnitude reduction in aeroallergens: by the end of the 24 week study, 0% of the active group had detectable aeroallergens, compared to 29% of control group. Not only was the aeroallergen level reduced, but there was an extremely effective reduction in allergen on the surface of mattresses. In the active group the mattress surface allergen level reduced from a baseline median of 480 ng (range 40 – 18400) to 0 ng (0 – 102) at six weeks ( $p < 0.0001$ ), which was maintained to the end of the 24 week trial. Whereas the control group mattress surface allergen levels only reduced by 53% to a median level at six weeks of 215 ng (no range is reported), which was also maintained to the end of the 24 week study. Clearly, there was a large magnitude reduction in allergens, however, which did not result in convincing improvements in asthma, which would cast considerable doubt on the allergen-reduction hypothesis, if only the allergen levels had been reported as concentrations. The allergen levels were only reported as ng, rather than as ng per g of dust, so it is possible that allergen load was well below the provocation threshold for the whole duration of the study.

Bearing in mind that the average allergen levels may have been low, a sub-analysis of Carswell and colleagues' bronchoprovocation results, among highly HDM sensitive

subjects, who had the highest baseline allergen levels and who also experienced a reduction in these levels, showed that these subjects did not experience a greater therapeutic effect, and in fact they had a similar absence of improvement in their bronchoprovocation as the control group [89].

#### **2.8.4 Dose-response relationship**

A dose-response relationship is one of the Bradford Hill criteria [73] for proving that a statistical association is representative of a causal association. A few publications provided a statistical measurement of the dose-response relationship between the magnitude of the reduction in allergen (“the dose”), and the size of the improvement in asthma (“the response”).

A study of air filters reported a formal statistical test of the relationship between the amount of dust collected in the filters, and the size of the reduction in peak flow variability, and found that it was highly significant for the amount dust collected by filters in living-rooms (for dust in pre-filters  $r=0.431$ ,  $p=0.005$ , for dust in all three filters  $r=0.356$ ,  $p=0.017$ ), interestingly this association was stronger (an  $r$  closer to 0) but not as statistically significant for the amount of Derp1 captured ( $r=0.292$ ;  $p=0.047$ ) [104]. This suggests that there may have been other allergens in addition to Derp1 that were being captured by the filters and which had a positive influence on peak flow variability. However, it is important to bear in mind that the actual  $p$ -values for this association would be much higher if they were adjusted for multiple comparisons. Strictly speaking,

they should be adjusted because they were not the primary endpoint, and the authors did test a number of associations. The primary endpoint was the inter-group change in peak flow-variability, which was not statistically significant.

Only a minority of publications provide a statistical test of the correlation between allergen-reduction and asthma outcomes, and they do not always provide sufficient data for the reader to undertake the calculation himself. These studies either do not report the values for these variables at all, or they report them as a graphic rather than as numbers. For example, the substantial and significant reduction in allergen levels which was accompanied by improved asthma in the 1999 study by Carswell et al only reported final allergen levels in box-and-whiskers plots [90].

A dose response relationship between PEFr and allergen-reduction was seen in the active group in study by Hayden et al, but it cannot have been causal. When a sub-analysis was performed among subjects, who should have been more capable of demonstrating an improvement in asthma from allergen-reduction (subjects who were sensitised to HDM allergens, had high levels of allergen at baseline, which reduced by >3-fold), subjects in the placebo group, who experienced the same large allergen-reduction as the active group, did not experience the same improvement in asthma. So although there was a dose-response relationship between allergen-reduction within the active group, a dose-response relationship was not evident in the subjects in the placebo group that had had the same allergen-reductions. This shows the value of having a control group to identify the possibility of a placebo effect [95]. Something other than the reduction in allergen must

have caused the improved asthma. In a similar vein, the trial by Marks and colleagues had a substantial Derp1 reduction at two weeks (61%) in the placebo group, but a smaller 29% reduction in the active group, yet there was no improvement in asthma in either group, (however, two weeks is only a short duration of allergen-reduction and allergens increased thereafter in both groups which might explain the lack of improvement in asthma) [100].

It can be concluded that there is no clear evidence of a dose-response relationship from the trials in this review.

### **2.8.5 Failure to reduce allergen levels equals negative improvement in asthma**

As hypothesised, studies that did not reduce allergens (or did not reduce them below the threshold for provocation), did not improve asthma.

A study of spraying natamycin onto the exterior of mattresses to kill the fungi that help keep HDM alive, found no effect on asthma, but also did not reduce HDM allergens either [102]. However, this study may have been limited by the inability of the fungicide to penetrate through the outside mattress covering to where the fungi and HDM are located. Perhaps the natamycin would have worked if the beds had been soaked in it, and then dried.

Marks and colleagues found no improvement in asthma (either intra-group or inter-group) in their study of encasings plus acaricide, but this is not surprising because the significant inter-group differences in allergen reduction at two weeks was short-lived and by three months both groups had similarly high levels of Derp1 [100].

Sette and colleagues also detected no improvement in asthma with benzyl benzoate and a holiday at high altitude, but this does not disprove the allergen-reduction hypothesis, because there were no reductions in allergens in their beds at home [105].

It is very difficult to reduce allergen levels - even after intensive professional cleaning and insecticidal bait, less than half of homes had their cockroach allergen levels reduced below the 8 U/g concentration threshold for exacerbating asthma symptoms, and it is unlikely that subjects' asthma improved in the trial by McConnell and colleagues (asthma outcomes were not reported) [101].

### **2.8.6 Significant allergen-reduction but little improvement in asthma**

Counter to the allergen-reduction hypothesis, some studies that did achieve statistically significant reductions in allergens did not improve asthma, or improved only some aspects of asthma, but not all aspects. It is not biologically plausible that only one or two elements of asthma would improve but not others, particularly if they have a related biological mechanism (such as PEF<sub>R</sub> and FEV<sub>1</sub>)<sup>III</sup>.

Although both the active and control groups experienced substantial reductions in HDM allergen in the study by Hayden, the only asthma outcome that improved was the active group's peak flow, there were no improvements in FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, or methacholine PD<sub>20</sub> [95]. This lack of consistency between the different asthma outcome measures suggests that the improvement in the PEFr cannot be interpreted as an improvement in asthma in general. This negative interpretation is consistent with the other deficits in this trial that have already been discussed. It is much more likely that the improvement in peak flow was due to the Pygmalion Effect / Placebo Effect because the active group received much more intensive much more “convincing” interventions than the control group (only the active group had their carpet removed, only the active group had a urethane layer in their bedding encasings).

There was a significant reduction in HDM allergen in Rijssenbeek-Nouwens and colleagues' active group compared to the control group, and this reduction was maintained long-term for the 12-month duration of the trial, however there was no statistically significant improvement in pulmonary symptoms, PD<sub>20</sub>, or other measures of asthma [56]. This outcome is counter to the allergen-reduction hypothesis, however a possible explanation for this unexpected result is that the baseline levels of Derp1 were extremely low, below the putative thresholds for sensitisation (2µg/g) and provocation (10µg/g), and therefore it is possible to argue that the baseline levels were too low to provoke asthma, and therefore there was nothing further to be gained from any reduction in the allergens.

Warner and colleagues also had a statistically significant reduction in Derp1 with air ionisers, which led to worse, not better asthma (night-time cough worsened), but they do not report if the levels of Derp1 fell below provocative thresholds [106].

Carswell and colleagues reveal that significant and sustained reduction in HDM and cat allergen in the air and mattresses to levels below the provocative dose, caused some extremely small improvements in a range of markers of asthma, however, given the small size of these improvements, and the fact that they occurred at some time-points but not at others, suggests that these ‘improvements’ were not real (not biologically plausible, not statistically significant), but were actually just random variations detected by multiple comparisons. For instance, there were statistically significant improvements at two weeks and 24 weeks, but none at six weeks, which is more plausibly explained as a Type 2 error from multiple comparisons (multiple outcome measures and multiple time-points at which these outcomes were measured) than evidence that allergen reduction improves asthma. Notably, the subjects in this study were particularly sensitive to HDMs, with a median skin prick test diameter of 5.5mm, and hence were ideally placed to show an improvement from the substantial reduction in allergen, if the hypothesis is true, so the fact that they did not improve, counts strongly against the hypothesis [89, 90].

Dietemann and colleagues also produced statistically significant reductions in HDM allergens in the active group that improved asthma within this group (baseline cf. end-of-study), but did not improve asthma compared to the control group. This negative finding could be explained by the argument that children spend so much time in bed then unless

allergens in the bed are reduced then asthma is unlikely to improve, but because only the HDM allergens in the carpets and soft furnishings was reduced, and allergens in the mattress were unchanged, then it is not surprising that there was no improvement in asthma [92].

### **2.8.7 Significant allergen-reduction with an improvement in asthma**

Only a minority of studies supported the allergen-reduction hypothesis and usually there was only an improvement in some, but not all, markers of asthma. Table 3 illustrates that 18 of 21 trials had a reduction in allergens (but five of the 18 were questionable), and only five of the 18 trials that reduced allergens significantly also consistently improved more than one asthma outcome, whereas the other 13 produced no change, or minimal improvement, or a negative change in asthma.

The biggest improvements in asthma occurred in the trials by Morgan and colleagues [66], and Krieger and colleagues [98, 99]. The former study measured allergens, but the later did not; the former was non-placebo controlled and the later was placebo-controlled. Hence, neither of these trials can irrefutably demonstrate that the interventions and allergen-reduction were the major cause of any improvement in asthma.

Ehnert and colleagues showed statistically significant reduced bronchial hyperresponsiveness in subjects whose HDM allergen levels decreased (those treated with mattress encasings), but not in subjects who allergen levels did not decrease (those

treated with benzyl benzoate or placebo) [94]. The improvement in PD<sub>20</sub> is likely to have been clinically significant, because the PD<sub>20</sub> increased by a large amount (4.5 times) [94].

Improved PEF<sub>R</sub> occurred at the same time that allergen levels were decreasing in the multiple intervention trial by Hayden et al (although other features of asthma control did not improve) [95].

Carswell and colleagues found an improvement in some, but not all asthma outcomes, as a result of demonstrable reduction in HDM allergen and cat allergen in mattresses, and a reduction in airborne mite allergens. However, the outcomes that improved only improved by an amount that would not be sufficient to be clinically relevant [89, 90, 103]. For instance, the FEV<sub>1</sub> (as a % of predicted) increased by 2.2% and decreased by 3.2% in the active and control groups, respectively.

Nishioka and colleagues witnessed a greater allergen reduction in the active group which was accompanied by significantly fewer asthma attacks (but this occurred in both atopic and non-atopic subjects) [108].

One can conclude from the discussion above that the trials that demonstrated large respiratory improvements from allergen-reduction were only a small minority among the trials reviewed, and their conclusions were subject to several caveats imposed by deficiencies in their study designs.

### **2.8.8 Cumulative dose of the intervention**

Some studies only had short-term one-off interventions, which were unlikely to produce lasting effects on allergen-reduction, and therefore it is not surprising that these studies showed no improvement in asthma after “allergen-reduction” interventions were instituted. For example, the study by Sette sprayed acaricide onto mattresses in children’s homes, but then took them to the Italian Alps for most of the duration of the nine month study, so that they were only exposed to their beds at home for a total of 30 days out of the nine month study period [105]. The study of ionizers to remove airborne HDM allergen was of only six weeks duration [106]. Trials that required subjects to regularly use an acaricide spray may have only had a small cumulative dose of this intervention if subjects were not compliant with using the spray, however there is no reliable method to measure compliance with this intervention.

## ***2.9 Possible reasons why some studies did not support the hypothesis***

### **2.9.1.1 Very low baseline allergens: not much room for improvement**

Eggleston and colleagues noted that it is possible that the fact that the improvement in asthma was so modest in their active group compared to the control group, may have been due to inadequate allergen-reduction. This in turn, may have been because of the

extremely low baseline HDM allergen levels, which were dramatically lower than the provocative dose of 10 µg/mg) [93]. As was mentioned in section 5.8.3 Rijssenbeek-Nouwens and colleagues also had very low baseline HDM allergens of only 0.97µg/g.

In contrast, some trials had high baseline allergen levels, which were significantly reduced, which was sometimes accompanied by improvements in some asthma outcomes. Two trials purposefully selected subjects who had high baseline allergen levels to give room for a large reduction [94, 106]. Ehnert and colleagues only enrolled subjects whose mattress Derp1 plus Derp2 levels were  $\geq 2$  µg/mg, which may explain why they had a significant reduction in allergen and improved bronchoprovocation thresholds [94]. Two other trials happened to have high baseline allergen levels. The majority of Hayden and colleagues' subjects had elevated baseline allergens, which reduced by more than three-fold, and led to improved PEFr at three and six months but no improvement in FEV<sub>1</sub> or bronchoprovocation, and increased hospital admissions (but admissions were not statistically significant in this small sample) [95]. The need for high baseline allergen levels in order for an intervention to demonstrate a significant effect was also seen in the study by McConnell et al [101]. In their study, subjects who had higher baseline cockroach allergen levels had a statistically significant greater reduction in allergen levels than those who had low baseline cockroach levels. However, although Warner and colleagues purposefully restricted enrolment to those whose living room and bedroom had  $>2$  ng/m<sup>3</sup> air-borne Derp1, which may have enabled their ioniser to produce statistically significant reductions in airborne allergen, this did not result in improved asthma [106].

Trials have not consistently supported the existence of an association of high baseline allergen level and large reductions in allergen, with improved asthma, and therefore the link between allergen-reduction and asthma control, must involve other additional causal factors which are powerful enough to negate any putative effect of allergen reduction.

### **2.9.1.2 Need to lower allergens in all environments children are in**

Although many trials in this review intervened to reduce allergens in a range of locations in the home, they did not reduce levels outside of the home, for instance Warner et al [106] refer to a Norwegian study that showed that children are exposed to a considerable amount of domestic allergens at school, and thus asthma may not improve if allergens are only reduced within the home environment [132-134].

### **2.9.1.3 Sensitised to allergens that are not part of the reduction interventions**

In van der Heide and colleagues' study, subjects were sensitised to several allergens, not just to HDM allergens, however the interventions were mainly directed toward reducing HDM, which might explain why subjects who got both active filters and mattress encasings did not reduce their eosinophil levels, as much as subjects who had placebo filters and active encasings [104]. Likewise, 23/28 active and 26/34 placebo subjects

were sensitised to grass (SPT  $\geq 3$ mm) in Carswell's trial, so unmitigated exposure to grass pollen may have reduced the beneficial effect of the other allergen-reduction interventions, so that the active groups asthma only improved modestly [89].

#### **2.9.1.4 Not sufficiently sensitised to the allergens under investigation**

Some studies did not measure allergen sensitisation as part of their inclusion criteria (yet it is only biologically plausible that sensitised subjects' asthma could improve from allergen-reduction), and not all studies that measured subjects' sensitivities reported whether or not there was a correlation between higher sensitivity and greater symptomatic improvement from allergen-reduction. For example, only outcomes for the whole sample were reported by Williams et al, yet 42% of subjects were not sensitised to HDM, and 64% were not sensitised to cockroach allergens [112]. Less than half of the active subjects (23/50) were sensitive to HDM in Eggleston and colleagues' study, although sub-group analysis of mite sensitive subjects found no greater effect size for the interventions in more sensitised children [93]. Only 62.8% of subjects in Morgan and colleagues' active group had a positive SPT for HDM [66]. Only 22/45 subjects in the van der Heide study were SPT positive for HDM and the wheal diameters were not reported [104]. Only 9/18 active subjects and 6/10 placebo subjects were HDM sensitive on RAST testing in the study by Hayden and colleagues [95]. Only 66.7% and 40.6% of active and control group subjects had positive RAST tests to any of the allergens, and only 33.3% and 31.3% were positive to moulds but mould was the primary target of the interventions in Kercksmar and colleagues' trial [96]. Boner et al; Krieger et al; Marks et

al; Reiser et al; Rijssenbeek-Nouwens et al, and Sette and colleagues conducted SPT as part of their baseline testing but did not report the results [98-100, 102, 105, 110].

There is evidence, albeit limited, that variability in strength of association between reduced allergens and improved asthma, may have been caused by variation in the degree to which subjects were sensitized to the allergens. For example, two studies had a  $\geq 5$ mm wheal as an inclusion criteria for SPT to HDM [89, 107], and two studies used 3mm as the wheal size [94, 106], whereas one study used a 2mm wheal size (2mm greater than the control weal) [66]. The median wheal diameter in the study by Carswell was 5.5 (range 3 to 11 mm) [89]. The two studies with the largest SPT ( $\geq 5$ mm) for HDM did not show a substantially greater effect of allergen-reduction; in the study by Warner et al there was only a non-significant trend to improved PC<sub>20</sub> in the group with MVHR, but the p-value of 0.085 for this may be due to the small sample size of only 40 subjects [107]. Likewise, the small sample size of 49 subjects with complete data may explain why Carswell et al observed only modest improvements in bronchoprovocation, despite enrolling very sensitised subjects, and despite performing sub-analyses of subjects who had high baseline allergens and whose levels fell the most [89].

### **2.9.1.5 Severity of asthma – too mild, or too long-standing?**

It is biologically plausible to hypothesise that only children with the most severe persistent asthma will respond to allergen-reduction. Most studies enrolled children with a variety of asthma severities, and did not always do a sub-analysis by asthma severity.

For example, Reiser and colleagues enrolled subjects with the full range of GINA severities [102].

Carswell et al [89] claim that their subjects had sufficiently severe asthma to show a causal relationship, on the basis that their inclusion criteria required that children had to have suffered more than one asthma attack in the previous year and have been breathless in at least one attack.

Insufficient severity of asthma does not explain why there was only a modest improvement in asthma symptoms in Eggleston and colleagues' study of comprehensive allergen-reduction interventions, because sub-group analysis of more severe asthmatic subjects, found no greater effect-size for the interventions [93]. Indeed, it is biologically plausible to argue that allergen reduction would only assist children *before* they ever get asthma, because longstanding asthma causes permanent airway remodelling, the symptoms of which cannot be ameliorated by allergen-reduction [135]. Hence, it is plausible to argue that it was not because children's asthma was not severe enough, that they did not improve after allergen-reduction, but actually it was because their asthma was too severe, and had a substantial irreversible component.

### **2.9.1.6 Failure to maintain zero change in allergens in the control group**

In some trials, the allergen levels in the control group reduced, which may possibly have occurred as a result of the placebo given to them (see sections 5.5.3 and 5.5.4 above).

The allergen-reduction hypothesis predicts that if allergens decrease in the control group, this would reduce the difference in effect size between the two groups, which would necessitate a larger sample size to show that the reduction in the active group was statistically significantly larger. Conversely, if the control group is given a placebo, and the allergen levels rise in this group, but they stay the same in the active group, then the difference between the groups cannot be interpreted as evidence that the active interventions reduced allergen levels, because there are two other possible interpretations. First, it may simply suggest that the placebo increased the levels. Alternatively, it could mean that the active interventions were effective in preventing the rise in allergens that occurred in the placebo group.

Marks et al used a placebo spray (but no placebo encasing), and found that allergen levels reduced in both active and control subjects' beds, and that there was a modestly significantly greater Derp1 reduction in the placebo group at two weeks which was non-significant by three months, which may explain why there was no significant difference in lung function between the two groups [100]. Allergen levels also reduced substantially in the placebo group in Dietemann and colleagues' study, (although this was only statistically significant in terms of guanine (not Derp1 or Derf1)), which one might argue is the reason why many asthma outcomes improved substantially in both the active and placebo groups, and why there were no statistically significant differences in asthma outcomes in the active group compared to the control group [92].

The study by Carter and colleagues should have been able to assess the independent effect of having a researcher visit the home of the subjects because it had a three-way parallel-group study design: 1. the active group got allergen-reduction interventions and home visits, 2. the placebo control group got placebo allergen-reduction interventions and home visits, and 3. a non-placebo control group got no interventions and minimal home visits [91]. However, because mite allergens reduced in both the placebo-control group and the active group and were not measured in the non-placebo control group, it is not possible to determine if the non-placebo-control group's poorer outcome was due to the fact that they did not receive as many home visits, or that they did not receive a placebo, or because they may have had higher allergen levels at the end of the study compared to the other two groups. Reduction in acute healthcare visits and reduction in allergens occurred in both the active group and placebo-control group and were not statistically different between those two groups. The reduction in acute healthcare visits in the active group was significantly different compared to the non-placebo control group. The fact that the reduction in acute health-care visits in the active group was not statistically significantly different to the placebo control group, and was only significantly different to the non-placebo-controlled group, suggests that the improvement may have been caused by either the placebo effect, or the increased number of home visits, or to differential allergen levels (but we do not know what the allergen levels were in the non-placebo control group). We can conclude that an inter-group comparison in this study only gives an uncertain picture due to the lack of allergen level data in the non-placebo control group. This study is consistent with the view that health care visits improve asthma, and

that the mechanism by which the visits do so, is a psychological rather than allergen-reduction mechanism.

Two trials by Carswell and colleagues, and Hayden and colleagues, demonstrated that a reduction in allergen in the control group does not always completely mask the effect of allergen-reduction in the active group, as long as the reduction in allergens is still significantly greater in the active group [89, 95].

The control group in the study by Carswell and colleagues [89] received comprehensive placebos and had a substantial reduction of 53% in Derp1 on the surface of mattresses to a median of 215 ng, whereas the active group reduced by 100% to a median level of < 2 ng. Likewise, there was a statistically significant reduction in the airborne Derp1 collected in Petri dishes at six weeks in the control group. The active group had statistically significant better FEV<sub>1</sub>, and lower total symptoms, and need for medications, but no improvements in several other asthma outcomes (see Table 3).

The reduction in allergen levels in Hayden and colleagues' [95] placebo group was small enough to not fully mask the improvement in asthma in active compared to controls, but may explain why the improvement was only modest: seven of ten placebo subjects had bedding HDM allergen levels >2 µg/g initially, and allergens reduced in four of these subjects' beds, although only one of these reduced below 2 µg/g. Figure 1 in their paper shows that although allergens reduced below 10 µg/g in quite a few placebo subjects' beds, they reduced below this threshold in many more subjects in the active group than

the control group. There was also a 3-fold drop in carpet allergen in 5/7 placebo homes. The allergen levels in the control group may have reduced because prior to enrolment they may not have laundered as often as was in the protocol and may not have used clothes driers as often (heat from driers kills mites), and some broke the protocol and used the active (hot water) intervention.

### ***2.10 Consistent evidence that some interventions do not work***

None of the studies found Acarosan (a benzyl benzoate acaricide) to be very effective when applied to carpets, although Dietemann and colleagues provided some evidence that Acarosan may potentially work in mattresses that have low baseline levels of allergens.

Cockroach bait did not work in two studies, which found no significant difference in allergen levels and/or cockroach counts in the active versus control groups [91, 98]. The only study that achieved a reduction in cockroach counts and allergens did not use bait on its own, but also utilised a range of other interventions (sealing cracks, application of insecticide, and thorough professional cleaning [101, 123]).

The active air-cleaners cancelled out the improved eosinophilia that occurred with active mattress encasings [104], which suggests that air filtration is not helpful. This is consistent with a systematic review that found only subjective, but not objective improvements with air filtration [87]; and a systematic review that found minimal benefit

of air filters and concluded that there was insufficient evidence to recommend air filters in domestic homes [136].

### ***2.11 Home visits ± education ± placebo-effect ± Hawthorne-effect***

Community-based allergen-reduction trials necessarily require that researchers visit the subjects' homes. Researchers must visit subjects' homes at least twice: once at the beginning of the trial to deliver the interventions and explain how to use them, and once at the end of the trial to collect outcome data. Most trials had multiple home visits, and in many studies, the home visits were also an opportunity to provide education about asthma, and psychological support regarding stressors (such as financial problems, and domestic violence). It is entirely plausible that the reductions in allergen levels, and improvements in asthma, were not caused by the allergen-reduction interventions (such as acaricides, impermeable covers), but were caused by the psychological support that was given to participants by researchers when they educated them about asthma and allergen avoidance during the home visits. It is also possible that the allergen-reduction was merely a confounding variable, and that the education and psychological support improved asthma by a mechanism that was independent of the reductions in allergen loads (such as the placebo effect).

Evidence about whether the placebo-effect is at work in allergen-reduction trials, can be gained by, firstly, using a factorial design in which a non-placebo-controlled group can be compared with a placebo-controlled group, and minimal visits can be compared to

intensive visits; and secondly, by measuring allergen levels in all groups in the factorial design.

Ten trials in this review were consistent with the hypothesis that psychological processes, rather than allergen-reduction, were responsible for improvements in children's asthma. Two trials were consistent with the hypothesis that the putative allergen-reduction interventions were not responsible for the lowered levels of allergens. These trials are discussed below.

The 1993 study by Boner and colleagues (which was identified by this review's search strategy), did not show a dramatic improvement in asthma after children holidayed in the Italian Alps, however some improvements were observed in similar studies by Boner and colleagues published in 1985 [33]. In the 1985 iteration of the "alpine holiday" therapy, there were improvements in bronchial reactivity and exercise tolerance and reduced medication usage. Just as in the 1993 study, the 1985 study did not have a control group, and so it is not possible to rule out the very plausible hypothesis that in fact the mechanism for the improved asthma was not allergen-reduction, but rather the emotional benefit to the children from being on holiday for an extended time.

The study by Carter and colleagues [91] is consistent with the view that health care visits improve asthma, and that this may work through a psychological rather than allergen-reduction mechanism (see Section 5.9.1.6 above).

The study by Hayden et al [95] also supports the conjecture that home visits are what caused the allergen-reduction, rather than the allergen-reduction interventions. In their study, the amount of allergen-reduction achieved in the active group, was similar to the reduction achieved by the placebo group (both achieved a massive >3-fold reduction). This 3-fold fall in carpet and bedding allergen in placebo homes occurred despite the fact that the placebo group received no intervention against carpet HDM, and the placebo encasings for the bedding were allergen-permeable. Surprisingly, although both groups had similar reductions in allergen, the active group had improved PEF compared to the control group (although there was no significant difference in FEV<sub>1</sub> or methacholine PD<sub>20</sub>). Four children in the active group compared to two in the placebo group were hospitalised. It is difficult to interpret this study because it had such a small sample size and p-values are only given for the PEF outcomes (however they are not adjusted for multiple comparisons), and the improvement in PEF is not consistent with the absence of improvement in other asthma outcomes. The most rational way to interpret this study is to conclude that there was no difference in asthma outcomes between the groups, and that the improvements in both groups were caused by the placebo effect (from receiving the interventions) and the Pygmalion and Hawthorne Effects (from receiving the home visits).

Dietemann and colleagues observed a significant improvement in symptoms scores in their active group, yet this group did not have a greater reduction in allergens compared to the placebo group (both groups had significant allergen-reductions), which suggests

that some other factor, such as a psychological factor, may be responsible for symptomatic improvements [92].

In Kercksmar and colleagues' study of mould remediation [96], children in both groups showed improvement in the number of asthma symptom days during the pre-remediation portion of the study, which suggests that the remediation was not responsible for all of the improvement in asthma, but that at least some of the improvement was due to things that occurred prior to remediation: contact with researchers, education about asthma and the need to use asthma-action plans, problem solving skills, the Pygmalion effect, *inter alia*. However, it is unlikely that all of the improvement in asthma was caused by pre-remediation contact factors, because of the fact that after some time, the improvement in the active group outstripped the control group, which suggests that although the positive impact of pre-remediation education and contact was effective, its efficacy was short-lived.

The findings of a Japanese study potentially support the argument that education at home visits rather than allergen-avoidance are the cause of improved asthma. This study compared intensive education and advice on allergen avoidance at a home visit (active group) to brief education and advice at a regular clinic visit, and divided the active group into atopic (A) and non-atopic (B), and the control group also into atopic (C) and non-atopic (D). Both the atopic and non-atopic children in the active groups (A and B) had a significant reduction, at the end-of-study (1 year) compared to baseline, in allergen levels, number of asthma attacks, and theophylline dose. Interestingly, even though the allergen

levels did not significantly decrease in the control group, both the atopic and non-atopic children in the control groups (C and D) experienced a substantially smaller, but still statistically significant reduction at the end-of-study compared to baseline in number of asthma attacks and theophylline dose. Analysis of covariance with 2 x 2 tables found a significant difference between clinic plus home-visit education, compared to clinic education without home-visit education, for a range of outcomes: HDM allergens ( $p < 0.001$ ); number of asthma attacks ( $p < 0.001$ ); and theophylline dose ( $p < 0.003$ ), but not for atopy. The fact that the non-atopic children responded identically to the atopic children, suggests that the improvements in asthma were not produced by an antigen-recognition immunologically mediated mechanism and therefore were unlikely to be the result of the reduction in allergen levels. Indeed, it is plausible to interpret these results, as evidence that supratentorial effects of education and home visits (Hawthorne/Pygmalion effects and also may have improved compliance with asthma action plans, use of medication, and avoidance of tobacco smoke) may have been caused at least some of the improvement in asthma (if not all of it), and allergen-reduction may not have been the primary cause [108].

A study of comprehensive HDM allergen-reduction, which used a double-blind randomised placebo-controlled design and was therefore capable of testing the allergen-reduction hypothesis, showed that even though only the active group had a significant reduction in allergen levels, there was no statistically significant difference between the active and control groups in terms of quality of life, asthma symptoms, peak flow, or use

of bronchodilators. This quite thoroughly shows that allergen-reduction did not cause the improvements in asthma, and therefore it is likely to have been a placebo effect [56].

The Hawthorne and/or Pygmalion Effects may explain why there was an improvement in the asthma of William and colleagues' control group, during the time that they were being observed *before* they got their intervention [112]. In fact the asthma severity scores for both groups improved sharply during the first four months of the study, then the stabilised at the lower levels, and at no time were the scores statistically different between the two groups.

The power of the placebo-effect can be seen in the study by Carswell and colleagues in which just as many parents in the placebo group thought their children had benefited from the trial, as the number of parents in the active group who thought their child had benefited [89].

Morgan and colleagues' trial is consistent with both the hypothesis that the 'allergen-reduction' interventions did not cause the reduction in allergens, and that the reduction in allergens did not cause the improvement in asthma [66]. Their control group received nothing other than regular visits to collect outcome data, yet they experienced large reductions in allergen levels, and significant improvements in asthma, which suggests that something about visiting people's homes, can cause a reduction in allergen levels. While it might very well be true that the statistically significant improvements in asthma in the active group compared to the control group was caused by the significant

difference in allergen-levels between the two groups throughout the study, it is also possible that the greater improvement in asthma in the active group compared to the control group was due to the placebo effect, because the control group were given no placebo. It is also possible that the improvements in asthma in the active group were due to the Hawthorne/Pygmalion Effects, because they had more home visits than the control group. Although the interventions teams were not clinically trained and were prohibited from discussing the medical management of asthma with the families, this is unlikely to have limited the Hawthorne/Pygmalion effects, because it is unlikely that the intervention teams would have been able to refuse to provide clinical advice and education if the subjects asked them for it.

Further doubt is cast upon the notion that allergen-reduction interventions cause the reduction in allergens, by the observation of Marks and colleagues that allergen reductions were substantially greater in the placebo group than the active group. In their study, two weeks after the interventions were installed, there was a substantially greater (61%) reduction in the placebo group compared to the active group, whose allergens reduction was much smaller (29%) [100].

It is not possible to tease out the effect of thorough education and contact between subjects and researchers at home visits, from the effects of the allergen-reduction interventions, on outcome measures, because in most studies these two potential causal factors are intertwined. It may very well be the case that, education and home-visits are a necessary precursor to effective allergen-reduction, and that perhaps without these home

visits, subjects will not take the time to thoroughly comply with the allergen-reduction interventions. Collectively, the trials in the present review give strong evidence that psychological processes are probably responsible for a considerable amount of the improvements in asthma that are witnessed in allergen-reduction research.

## **2.12 Statistical issues**

### **2.12.1 Intention-to-treat analysis versus as-treated analysis**

In some respects, it makes more sense to analyse a trial's results in terms of whether or not subjects actually received the intervention. After all, it is only biologically plausible that the interventions could alter the outcome if and only if the interventions were delivered. However, analysing the results in this way can introduce all the flaws that researchers intend to avoid by using randomised subject allocation. This was an issue for the study by Kercksmar and colleagues whose results were non-significant in an intention-to-treat-analysis but were significant in an as-treated-analysis [96].

### **2.12.2 Adjustment for multiple comparisons**

None of the studies made any adjustment for multiple comparisons, yet trials investigated a myriad of outcome measures, and repeated each of these outcome measures at multiple time-points, which presents a serious risk of making a Type One error (falsely rejecting

the null hypothesis). Not only did studies measure many different possible outcome measures, they also investigated a multitude of potential causal factors in order to find one that did have a significant association with asthma. Moreover, a number of studies also utilised a number of different ways of measuring the same causal/outcome factor. Furthermore, some studies performed multiple ways of analysing the same data.

For example, Carswell and colleagues [89] assessed the outcome measure of bronchoprovocation in two ways: both as Bronchial Hyper-Responsiveness, and as Mean Dose Response Slope; and they also analysed allergen levels in two ways: both as ng and as ng per g of dust. In their study, there was no improvement in peak flow, but there was a small but statistically significant improvement in FEV<sub>1</sub> [89]. This same study reported a significant effect on asthma symptoms, if all the individual symptom scores were summed together into a total score, yet there were no significant differences between the two groups for the two key symptoms of asthma – wheeze and cough. It seems biologically implausible, that this total symptoms score would be statistically significant at two weeks and 24 weeks, but not at six weeks [89]. It seems implausible that active subjects would have experienced improved symptoms after just two weeks, only to get worse again at six weeks, and then improve by 24 weeks. This is likely to be the result of making multiple comparisons [89, 90, 103].

The issue of multiple comparisons arises when outcomes are measured at multiple time-points, for example, in the mould remediation study there were four different clinic visits

and four telephone follow-up calls, giving eight different time points where data could be collected [96].

The trial by Morgan and colleagues found the biggest impact of allergen-reduction on asthma outcomes, compared to other trials in the literature, however they made no adjustment for multiple comparisons, despite the fact that their trial examined one of the widest ranges of allergens and outcome measures in the literature [66]. Had they adjusted for multiple comparisons, their results may not have appeared so encouraging, particularly after factoring in the lack of a placebo intervention, and lack of blinding.

Multiple comparisons also occur when many different areas of the house are measured, and when outcomes are measured in several different ways, for example in a study of MVHR the Derp1 was measured in bedroom carpet, mattress, living room carpet, and sofas; and not only was each site's Derp1 measured as Derp1 per square metre, but also as Derp1 per gram of dust (the reduction per square metre was statistically significant in more locations) [107].

Multiple comparisons are a risk when a considerable number of questionnaires, scales, and psychological batteries are used, each with their own myriad questions and sub-scales. For example, Williams et al report very few positive findings, one of which simply related to a small sub-component, the "Functional Symptom Score", of a much larger scoring system that had many sub-components, and which was only analysed post hoc [112].

A further method of maximising the harvest of statistically significant results from an abundance of data, is to adjust the data for numerous different potential confounding factors until a positive association is found. For example, an association can be non-significant in univariate analysis, but significant in multivariate analysis that takes account of any number of potentially confounding variables. This was the case in the mould remediation study by Kerckmar et al who found there were no significant univariate associations with the number of symptoms days, but when the various causal factors were analysed together and adjusted for baseline asthma severity and seasonality, then the associations were significant [96].

There are at least two possible ways to deal with the problem of multiple-comparisons without the need to statistically adjust for it by lowering the p-value that is considered significant. One way, would be to establish summary measures to coalesce some individual outcome variables into one summed variable. Another approach would be to prioritise the outcome variables in terms of the magnitude of the effect they are likely to have on asthma (according to biologically plausible mechanisms). It would be quite a hard judgement-call to decide which outcomes to sum together, or which ones are likely to have the greatest effect on asthma – what is more plausibly related to asthma severity – FEV<sub>1</sub> or PEF; spirometry or symptoms? On the one hand spirometry is more accurate than self-reported symptoms and self-conducted PEF, but on the other hand spirometry only occurs at occasional intervals, and could miss detecting worsened lung function that occurs in-between clinic visits, whereas daily PEF and symptom reports are less likely to

miss short-term changes in asthma. A decision about prioritising the outcomes must be made before enrolling subjects, and must be published in a register of clinical trial designs.

## **2.13 Mixed effects**

### **2.13.1 Good for some outcome measures but not for others**

A visual summary of the outcomes of the studies in the present review are in Tables 2 and 3.

Not only does the assessment of a wide range of outcome measures lead to the problem of multiple comparisons mentioned above, it also creates the possibility that positive outcomes will be overly-emphasised without acknowledgement of the often more convincing negative outcomes. This can happen when researchers only publish the positive statistically significant outcomes and do not report their negative and non-significant findings (journals' word limits encourage this). Even if all the outcomes are included in the publication, the reader can (intentionally or unintentionally) cherry-pick the outcomes to suit his or her viewpoint.

For example, in the study by Dietemann [92], the outcomes that were significantly different between groups were: Derp1+Derf1 in various elements (excluding mattress and carpet), but there were no significant differences in asthma outcomes. The outcomes that were significantly different within groups were: Derp1+Derf1 (but not guanine) in various elements and carpets (but not mattresses), clinical score, visual score, FEV<sub>1</sub>, FEF

20-75 in the active group; and within the placebo group the significant differences were guanine in mattresses, visual score, FEV<sub>1</sub>, and FEF<sub>25-75</sub>. Neither group had significant improvements in mean morning or evening PEF<sub>R</sub> in the within-group or the between-group comparisons.

Boner and colleagues [110] found that the combination of staying at high altitude for periods of time, and use of acaricide on bedding during short stays in the low altitude homes, improved eosinophil markers of inflammation, but this did not translate into improved bronchoprovocation.

Carswell and colleagues [89, 90] also had improvements in some markers of asthma, but no change or a worsening in other markers of asthma. Their interventions produced significant reductions in HDM allergen in Casella samplers, Petri dishes, and bedding but not in bedroom carpets. These markers of asthma improved:

- improved FEV<sub>1</sub> from 102.7% (SD 5.8) at baseline to 105.0% (10.2) at 24 weeks in the active group; in the placebo group there was a decrease from 101.8% (11.8) to 98.6% (15.3) over the same period. This difference between treatments was significant at 24 weeks ( $p < 0.05$ );
- slightly greater reduction in the histamine dose response slope six weeks after intervention in the active group ( $p = 0.02$ );
- at 24 weeks active group had less bronchodilator use, and lower symptom scores.

These markers of asthma were unaffected:

- equally large increase in PEF for both active and control groups at six weeks (99.6% (SD 17.8) reduction in active group, 98.9% (SD 14.5) reduction in placebo group);
- pattern of change in PEF variability similar between groups;
- frequency of PEF recordings below the 95% confidence interval (< 87.6% predicted) for a normal child similar between groups;
- there was no sustained improvement in histamine dose response slope (at six months,  $P = 0.23$ ).

Some studies, which had multiple outcome measures of asthma, found seemingly contradictory results, such as improvements in some outcomes, but worsening in others, despite both outcomes sharing a similar biological mechanism. For example, the air filtration study by van der Heide had what seem to be contradictory results.

	<b>Peak flow variability</b>	<b>PD<sub>20</sub> histamine dose</b>	<b>Eosinophil counts</b>	<b>IgE levels</b>
<b>Group 1: active air-cleaners in living-rooms and bedrooms.</b>	<b>No change</b>	<b>No improvement</b>	<b>No significant change.</b>	<b>No change</b>
<b>Group 2: placebo air-cleaners plus allergen-impermeable mattress covers</b>	<b>Trend to lower values</b>	<b>Trend to improvement</b>	<b>No significant change. Trend to lower numbers.</b>	<b>Significant decrease.</b>
<b>Group 3: active air-cleaners plus allergen-impermeable mattress covers.</b>	<b>Trend to lower values</b>	<b>Significant improvement (i.e. increased PD<sub>20</sub>)</b>	<b>No significant change. Trend to lower numbers.</b>	<b>No change</b>

It seems contradictory that Group 2, compared to Group 3, would have a reduction in IgE levels, but only a trend to improved PD<sub>20</sub>, whereas Group 3 had the inverse effect (an improved PD<sub>20</sub> but no change in IgE levels). It would have been helpful if van der Heide et al had reported the levels of B cells, and mast cells, from which a better picture could have been drawn of the immunologic causal pathways. Part of the explanation for why the van der Heide study had mixed rather than clear-cut results from the air filters and mattress encasings, is explained by the multivariate regression analysis that they performed. This analysis found that the interventions only explained a small proportion of the variance in histamine PD<sub>20</sub> dose, and other factors played a role. In fact, 32% of the variance of the change in airway hyperresponsiveness (PD<sub>20</sub> histamine) between baseline and six months could be explained by the following four variable: treatment group ( $\beta=0.4039$ ;  $p=0.005$ );  $\Delta$ Derp1 in mattress dust between 0 and 6 months ( $\beta=0.4976$ ;  $p=0.002$ ); floor covering in living-room ( $\beta=-0.3393$ ;  $p=0.014$ ); and presence of cats/dogs ( $\beta=0.3661$ ;  $p=0.020$ ). So variability between the groups in terms of floor coverings, and cat ownership can obscure the effectiveness of air filters and mattress encasings. Thus studies of allergen-reduction need not only to address as large a range of allergens as possible, but also a wide variety of potential confounding factors, such as floor coverings and pets. However, this will require adjustment for multiple comparisons.

The differences in the age range of subjects (and variety of allergens to which they are sensitised) might explain why some studies found positive effects while others found no effect of some allergen-reduction interventions. For example, van der Heide et al [104] hypothesised that the fact that the subjects in a German study [94] of bedding encasings

were young children (who were exclusively sensitised to HDM), whereas the subjects in an Australian study [100] of these encasings were adults (who were sensitised to multiple allergens), explains why the former study found a positive improvement in asthma as a result of encasings, whereas the later study found no such effect.

In the paper by Eggleston and colleagues [93], Table 4 “Children Reporting Asthma Symptoms in the Past 2 Weeks” (see below), illustrates how allergen-reduction interventions can be beneficial for some asthma outcomes but not for others, and beneficial at some time-points but not others. This is evidence that there is a lack of consistency, which according to the Bradford Hill criteria, reduces the likelihood that the association is causal.

Table 4. Children Reporting Asthma Symptoms in the Past 2 Weeks\*

	Control group					Treatment group				
	Baseline	3 mo	6 mo	9 mo	12 mo	Baseline	3 mo	6 mo	9 mo	12 mo
Daytime symptoms	50	55	60	60	59	58	59	50†	58†	55‡
Symptoms with exercise	51	47	52	42	58	52	41	39†	31	33
Nighttime symptoms	30	41	42	32	31	42	41	30‡	40	30
Interfere with child's activity	60	49	48	42	41	71	57	40‡	51‡	49

\* Data are given as percentages.

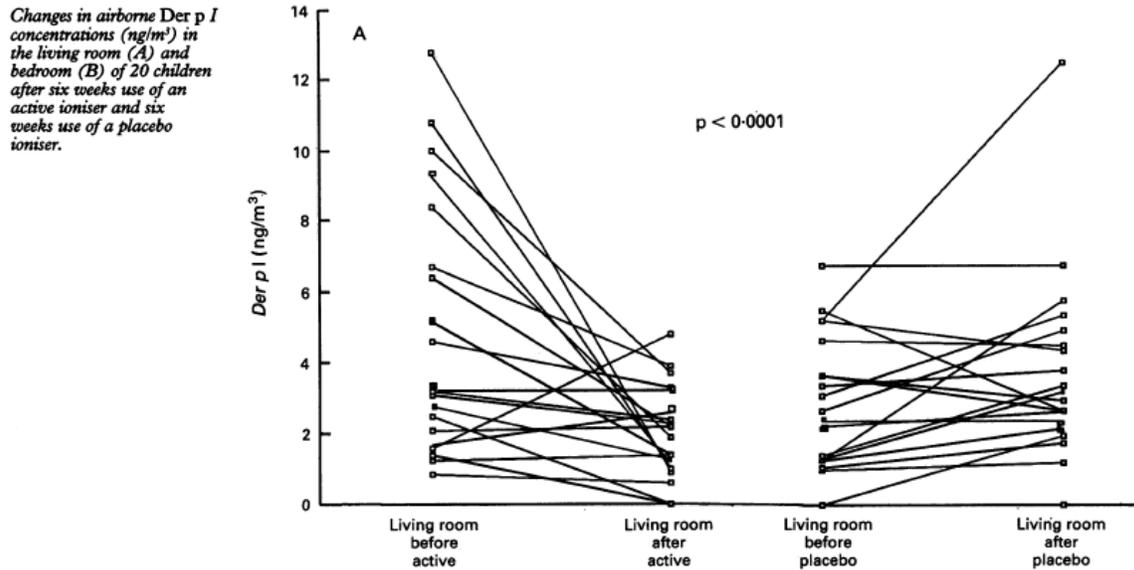
† P < .01, difference from baseline compared with control.

‡ P < .05, difference from baseline compared with control.

### 2.13.2 Good for some subjects but not for others

Like with many interventions in medicine, there are some people and environments that respond well to the intervention and there are those that do not. The use of means and medians can help to give an idea of whether most people did well or poorly, but the mean and median can mask the fact that a minority of subjects can have the opposite outcome

to what the majority experience. For example, in a study of air ionizers, although the majority of children had a reduction in airborne Derp1, some had an increase, as can be seen in the figure and table below [106].



**Table 1** Comparison of changes in airborne Der p I levels during active and placebo ioniser periods

	<i>Increased or unaltered Der p I</i>	<i>Decreased Der p I</i>
Active	11	29
Placebo	33	7

$p < 0.01$  ( $\chi^2$  test).

As predicted by the allergen-reduction hypothesis, only sensitised children would be able to have improved asthma from reductions in their exposure to allergens, and this was seen in the study by Carter and colleagues in which the correlation between decreased mite allergen and reduced acute health-care visits was only significant (it was highly significant  $p < 0.01$ ) for subjects who were sensitised, but not those who were not sensitised [91].

## **2.14 Some positive studies had only small magnitude improvements**

Many of the positive studies only showed small magnitude improvements in asthma, which would have little relevance clinically or in every-day life. The magnitude of a causal relationship is one of the Bradford Hill criteria: the greater the magnitude of the effect size, the greater the plausibility that the statistical association is a causal association. Doubt remains about the existence of a causal relationship, when studies have few positive findings or findings of very low magnitude. For example, the study reported by Carswell et al [89] found that improvements in PEF were only statistically significantly different between the active and control groups at 24 weeks, yet at this time the differences between the two groups were very small: an increase from 102.7% (SD 5.8) at baseline to 105.0% (10.2) at 24 weeks in the active group; compared to a decrease in the placebo group from 101.8% (11.8) to 98.6% (15.3) over the same period. There were similarly modest, but statistically significant improvements in FEV<sub>1</sub> from 102.7% to 105.0% at 24 weeks in the active group. Likewise, the improvements in asthma in several other studies were only very small [66, 104]. Table 3 illustrates the large number of outcomes that did not change ( $\pm$ ) compared to those that improved (+) after allergen-reduction interventions.

## ***2.15 Most positive studies had only short-term improvements***

None of the studies had very long durations, so even if an improvement remained at the end of the study, that does not mean it would have continued long-term. For instance Weeks, Carswell and colleagues had significant reductions in HDM allergens on encasings at the end of their study, but it was of only three months duration [103]. It is not uncommon for trials of HDM allergen reduction to observe that after an initial reduction in allergen loads, the allergens quickly re-accumulate. For examples, Marks and colleagues observed that the initial reduction in HDM allergens in beds was statistically significant at two weeks, but was no longer significant at three months [100].

Some studies had a long enough follow-up period to find that the improvements were not durable. For instance, Carswell and colleagues found improvements in bronchoprovocation and daytime wheeze and cough were significant at six weeks but not at six months [89], even though the HDM allergens were still significantly lower in the active group at six months [103]. Although the significantly lower daytime symptom score in Eggleston and colleagues' active group remained throughout the entire twelve months, the improvements in other asthma outcomes were not significant at twelve months (symptoms with exercise, night-time symptoms, and interference with child's activities) [93]. The improvements in Ehnert and colleagues' active group's symptom-days were statistically significant at ten months, but were no longer significant 60 days later; and Emergency Department (ED) visits were significantly reduced at six months but not at twelve months [94].

## ***2.16 Incomplete, missing, withdrawals, and loss-to-follow-up***

There was a loss of subjects (through exclusion, withdrawal, and loss to follow-up) in many of the trials which, when combined with loss of data (data that was missing or that had to be discarded due to errors in its collection), left trials even more underpowered than they already were, and also may have introduced bias (if what was lost in one group was systematically different to what was lost in the other group).

Carswell and colleagues had lost eight out of 70 subjects between screening and delivery of interventions; a further eight subjects withdrew; acaricide powder was not applied in one home for fear of damaging the carpet, and was mistakenly not applied to a sofa in another home. Four subjects had to be excluded from the analysis because their bedding encasings were mistakenly removed before the end of the study. Two children did not attend the final clinic visit and did not complete their diary cards. Therefore there were only 51 out of the original 70 subjects who contributed to the full analysis [89, 90, 103].

Two of the 25 subjects who were randomised dropped out of Hayden and colleagues' study shortly after randomisation due to family problems [95].

Marks and colleagues enrolled 39 subjects, four withdrew prior to randomisation, two withdrew later; and the final post-intervention assessment data were missing for 5/35 subjects [100].

Three of the 51 of Reiser and colleagues' subjects dropped out due to non-attendance [102].

Of the 180 subjects Eggleston and colleagues enrolled, 51 refused to continue or were unable to be contacted, and 16 failed to attend scheduled visits [93].

Of the 38 subjects that Rijssenbeek-Nouwens and colleagues enrolled, eight did not complete the study: five from the placebo group [three because of unstable asthma (significantly more in placebo group), one moved city, one did not record symptoms or medication use]; and three from the active group [one because study was too burdensome, two did not fill in their diaries] [56].

There was 10% and 24% loss-to-follow-up in the active and control groups, respectively in Kercksmar and colleagues' study [96].

Attrition of subjects during the conduct of the study by Williams et al may have had a profound effect on the outcomes of the study, by selecting subjects of specific characteristics and thus biasing the study and reducing its external validity: 981 children were assessed as eligible, but only 410 were enrolled, and only 161 completed baseline activities [112]. Williams discusses elsewhere the reasons why allergen-reduction trials have substantial difficulties with subject retention: 43% did not attend study visits, retention rates were significantly higher for participants enrolled in the second year of the

study, for those who had lived at the same residence for longer, and for those who had enrolled during a face-to-face follow-up home visit, rather than at the emergency department [137].

Two studies explicitly mentioned that data were either missing or had had to be discarded. Sixteen percent of the active group did not receive the main intervention in the study by Eggleston and colleagues [93], and in the study by Carswell and colleagues 6/62 subjects did not receive the full intervention and were excluded from the analysis [90].

### ***2.17 Subject non-compliance / protocol violations***

Most authors reported that a small proportion of subjects did not comply with study protocols, and that there were also some protocol violations by research staff, which firstly reduced the amount of data that these studies were able to generate, and secondly, potentially introduced confounding and bias. The problem of poor compliance with recording daily peak flow has been raised by several researchers in the literature, and is a major hurdle for community trials [138, 139].

Two active group and two placebo group mattress encasings were removed before the last visit so the subsequent dust measurements were discarded. This meant there were 24 active and 27 placebo allergen results available at the end of this study [89, 103]. Eggleston et al had even fewer subjects comply with bedding encasings, so after one

year, only 27% of subjects actually had encasings in situ [93]. Several subjects in the placebo group actually laundered bedding using hot water (the active group's intervention) instead of cold water (the placebo intervention) in the trial by Hayden et al [95]. The inverse of this occurred in Carter and colleagues' trial in which only 50% of subjects were deemed to have complied with the allergen-avoidance interventions; a number of subjects in the active group used only warm water instead of hot water, and subjects in the placebo group had laundered bedding more intensively than was in the protocol – these violations are likely to confuse the outcome of the trial because they will increase allergens in the active group and decrease them in the control group [91]. Only 75% of the active group used the air cleaners in the study by Eggleston et al [93], and in van der Heide and colleagues' trial the noise made by air cleaners, may have been the reason why some subjects either did not operate them at full capacity, or had stopped operating them at all [104].

There was quite poor subject compliance with recording daily symptoms and peak flow measures in the study by Carter and colleagues, which is probably why they do not report these outcome measures [91]. Poor diary keeping also occurred in several other studies: in 8/35 subjects [100], 3/38 subjects [56], and was mentioned by Hayden et al (no numbers were given) [95].

Subjects moved from the homes that had been treated: 3/35 subjects moved house [100], 1/38 subject moved house [56], 23/180 subjects moved house at least once and three moved out of the area [93]

Of the 180 subjects enrolled by Eggleston and colleagues, 16 failed to attend scheduled visits, 49 changed their phone numbers [93], and only 97 completed the study [93].

Unlike most researchers, Krieger and colleagues had very good compliance, which they attributed to the esteem that subjects had for the community healthcare workers who conducted regular home visits, and this esteem must have been very high and unrelated to the number of clinic visits because there was no greater compliance in the active group that had four – eight visits compared to the control group that had one visit [98].

Some protocol violations were due to unavoidable practical issues, for example Kerckmar and colleagues were not able to remediate some homes that had been randomised to the active group, so six subjects were given the opposite intervention to the one they were randomised to in the study by Kerckmar and colleagues [96].

### ***2.18 Small sample: underpowered, unequal randomisation***

Generally the studies had extremely small sample sizes, which exposed them to the risks of being underpowered, and of unequal distribution of confounding factors despite randomisation. For instance, Boner and colleagues only enrolled twelve subjects.

The findings of several small studies intimated that they were underpowered, and had their sample size been bigger, they might have found statistically significant results. For

instance, Dietemann and colleagues only enrolled 26 subjects, and although they found statistically significant changes within groups, the changes were not significant between groups. Hayden and colleagues' enrolled only 25 subjects and witnessed a non-significant improvement in FEV<sub>1</sub> at six months, but to have had adequate power to detect this small improvement, would have necessitated a sample size of 4,698 subjects (power of 90% and alpha of 5%) [95].

Carswell and colleagues' trial clearly demonstrates the danger of unequal distribution of confounding factors between groups, due to a small sample size. Only 49 subjects remained at the end of their trial, seven of whom owned cats, yet six of them were in the active group active group [90].

Only three trials reported power calculations. Rijssenbeek-Nouwens and colleagues calculated a 99% power to detect a 20% increase in PC<sub>20</sub> histamine for their study of encasings and hot water laundering [56]. Williams and colleagues presented a power calculation, that 300 subjects were required for 80% power to detect 25% increase in PEF<sub>R</sub>, however only 161 were randomised, and only 34 completed the twelve month assessments. Warner and colleagues acknowledge in their discussion that their study of MVHR was underpowered, and they provide a power calculation to show this [107].

Although it is vital to have a large enough sample size, it is not easy to recruit subjects, or retain them. Kercksmar and colleagues study had difficulty recruiting enough subjects despite "aggressive recruitment measures" [96]. Dietemann and colleagues found it to

find enough people who had the right HDM sensitivity, and who were also willing to take part in a study that demands so much of their time for cleaning procedures [92].

In addition to the problem of recruiting and retaining enough subjects, there is the serious limiting factor of the high cost of allergen-reduction interventions, and visiting subjects' homes. The mean (SD) cost of remediating a home to remove and prevent mould was US\$ 3,458 (2,795) [96]. The cost of the mechanical ventilation heat recovery units was the reason cited by Warner et al for why they had to restrict their sample size, which meant that they were underpowered to detect small improvements in asthma control [107]. Cost was one of the reasons why a third arm, that would receive no visits from researchers was not added to a placebo-controlled study for the purpose of investigating the Hawthorne Effect [112]. Funding agencies might be enticed into funding allergen-reduction studies by the cost estimation of Krieger and colleagues, who calculated that the high cost of interventions were offset by savings of US\$189-\$721 per participant [98].

### ***2.19 Practical difficulties in the home environment***

The trials in this review showed that there were numerous hurdles and complications to overcome in the home environments of the subjects, which related to the complexity of working with people (particularly children and families), the complexity of the structure of the home, and the vicissitudes of the climate. In order to adequately surmount these difficulties, a multifactorial study, with several arms, and a large sample size, is required.

Examples of complexities of the physical home environment, within this review:

1. need to exclude children who sleep on the bottom half of a bunk-bed, need to treat both children's beds the same if they share a room, need to stop children sharing beds [89];
2. landlords refused permission for home remediation aimed at reducing sources of water leaks and dampness for two potential subjects in the study by Kercksmar and colleagues [96];
3. not all mould is visible, some is hidden behind the walls, or within the building materials (occult mould), and requires trained technicians to identify it;
4. six subjects could not use the acaricide, because it would damage their carpet [89];
5. the nature of the building can make it impossible to conduct some interventions, which means that many homes must be excluded from trials which reduces their external validity [107];
6. homes can vary in their humidity levels, which can be unequally distributed in trials that have a small sample, this can be ameliorated by using stratified randomisation (although this can be problematic in itself) [107].
7. there are so many potential confounding factors, and such huge variation in so many aspects of the home environment; for example, four subjects had quilted headboards, which had higher mite allergen levels than the mattresses [89].

Examples of complexities of dealing with people:

1. one subject changed his mattress after three months, and two subjects did not stay continuously in the treated homes, and these three subjects were withdrawn from the original sample of 26 [92];
2. two children were unable to continue with the study after randomisation because of “family problems” [95];
3. allergen-impermeable bedding encasings have to be removed from the mattress if the child has an episode of enuresis [95];
4. poor compliance with symptom diaries [95, 100];
5. two subjects in the control group were non-compliant with the request to wash laundry in cold water, and washed in hot water, which was the intervention for the active group [95];
6. four subjects moved homes [100];
7. six subjects were not well enough to perform histamine bronchoprovocation tests [100].

## ***2.20 Biological Plausibility: just due to natural variation?***

As is discussed in detail in Appendix 2, merely demonstrating that there is a statistical association between two factors is not sufficient to prove there is a causal association between these factors. Sir Austin Bradford Hill set out the other essential components that are required to prove causality, one of which is biological plausibility [73]. A consideration of the biological plausibility of the findings of several studies within this

review, suggests that there is not always a causal associations between first, the putative ‘allergen-reduction interventions’ and the subsequent reduction of allergen levels; and second, the reductions in allergen levels and the subsequent improvement in asthma. In this section, first the trials that do not seem biologically plausible are outlined, and then the trials that are plausible are presented.

In the study by Carswell and colleagues, although the active group had a statistically significantly lower average allergen level and better average asthma outcomes at the end of the study compared to the control group, there was not a statistically significant *correlation* between changes in asthma outcomes and changes in the allergen levels, which weakens the argument that this was a biologically plausible causal relationship (see Section 5.8.4 Dose-response relationship). Moreover, it is not biologically plausible that the improvements in bronchial lability were caused by reductions in allergens, because allergens reduced over the whole duration of the study, but the improvements in provocative dose were only statistically significant at six weeks and not at six months. Furthermore, there was a statistically significant difference between the active and placebo groups in terms of their total symptom scores at two weeks and 24 weeks but not at six weeks, yet allergen levels remained low throughout this period of time and certainly did not increase at six weeks and reduce again at 24 weeks [89]. Unlike the bronchial lability outcomes, the bronchodilator usage outcomes were biologically plausible. There was a statistically significant difference in bronchodilator use between the two groups at six months, but not at six weeks, which could be explained biologically on the basis that there may be a slow extinguishment of pro-inflammatory biological

pathways and chemicals, which could conceivably take at least six months to manifest itself in reduced bronchoconstriction.

Dietemann and colleagues observed a statistically significant greater improvement (end-of-study compared to baseline) in clinical scores in the active group, but not in the control group, yet both groups had similar objective (spirometry) asthma outcomes and similar reductions in allergen, which suggests that the clinical scores are not biologically plausible. As was discussed in Section 5.13.1 “Good for some outcome measures but not for others” van der Heide and colleagues found that filters+encasings improved the PD<sub>20</sub>, but not eosinophils and IgE levels, which seems to be biologically implausible [104].

The pattern of change from baseline, in levels of Derp1 that Carswell and colleagues found in petri dishes, does not seem to be plausibly caused by the intervention [89]. The changes in the active group appear to be plausible, until one looks at the change in the placebo group. The active group’s Derp1 levels decrease dramatically from baseline to two weeks, then reduce further at six weeks, but rise again at 24 weeks and are somewhat less statistically significant at 24 weeks compared to six weeks. In the placebo group, the levels go up between baseline and two weeks, then reduce by a large and statistically significant amount at six weeks, then increase slightly by the 24<sup>th</sup> week to levels that are no longer statistically significantly different from baseline and are similar to the levels found in the active group. This may merely be a reflection of the natural variation in allergen levels in the environment.

	Active		Placebo	
	Median	Range	Median	Range
Baseline	0.63	0.0 – 3.2	0.37	0.0 – 3.1
2	0.29	0.0 – 1.5 *	0.67	0.0 – 2.9
6	0.11	0.0 to 1.2 **	0.24	0.0 – 2.6 *
24	0.20	0.0 – 2.8 *	0.28	0.0 – 2.7
*P<0.05. **P<0.01				

Indeed, it is quite plausible that many of the changes in allergen levels that researchers report as being caused by their studies' interventions might actually just represent random variation inherent in repeat measurements at the same site in the same season, and by measurements made in different seasons. The year-to-year variation in Derp1 in the same homes varies from a median (5-95% range) of 1.35 (0.04 – 42.83) to 1.61 (0.08 – 62.58), and the Spearman rank correlation between these two time-points was  $r = 0.82$  [140]. These high natural variations in Derp1 have been replicated by several other groups. In Taiwanese mattresses, geometric mean Derp1 varied from 630 to 77,000 ng/gm in February and December, respectively [141]. In the Australian domestic climate, there are also large seasonal variations from 2.3 (95% CI 1.5-3.3) to 7.2 (95% CI 5.5-9.4)  $\mu\text{g/g}$  in mattresses [142], and even three-fold variations within the same fortnight [143].

Not only is there seasonal variation, but there is also substantial variation between Derp1 levels in repeat samples, that were taken at the same time-point. The Coefficient of

Variation (COV) for repeated measurements of Derp1 at a single point in time was 32.7% and 4.0% for in carpets and mattresses, respectively [144]. Mean COV for Derp1 and Feld1, collected at the same time-point from different areas of the same carpet were 53.1% (range: 28.5-136.8), and 65.6% (range: 28.5-131), respectively in Wellington [145, 146]. A six-year trial found that the correlation between allergen levels repeated in the same sites at different time-points varies considerably from  $r = 0.46$  for Derp1, to  $r = 0.21$  for Feld1 [147]. To take account of seasonal variation, the baseline and end-of-study measurements should be taken in the same season, and the seasonality of assessments must be similar for both groups in the study. To take account of variation within the same time-point multiple samples should be taken from each site every time samples are collected.

The fact that allergens reduced in several placebo-controlled groups, even though the placebo-interventions were supposed to be ineffectual, could be interpreted as further evidence that changes in allergen levels are caused by natural variation and are not caused by the trials' interventions [91]. However, it is also possible to interpret this as evidence that the placebo interventions and/or home visits were mildly effective in reducing allergens. The following paragraphs discuss several trials in which the changes in the placebo group's allergens suggest that the changes were not caused by the interventions.

The changes in allergen levels in the randomised placebo-controlled study by Hayden and colleagues [95], suggest that the multi-faceted interventions were not the causal agent of

the reductions, but some unknown factor was the cause. Allergen levels fell more than three-fold in five of the seven placebo subjects' homes, despite the fact that their bedroom carpet was not removed, and they sprayed their living-room carpet with water, whereas the active group had their bedroom carpet removed and they sprayed their living room carpet with tannic acid. Even if the control group vacuumed more often as a result of being in the study, this is unlikely to be the cause of the impressive reduction in allergen levels because the placebo group were not given HEPA filter vacuum cleaners. It is biologically plausible to hypothesise that the reduction in allergen levels in the placebo group (and indeed the active group) was caused by normal random variation in levels. The wide variation that naturally occurs in homes was also evident in the baseline levels of the active group before they received the intervention: 0.4 to 25  $\mu\text{g/g}$  on the bedroom floor, and 0.4 to 33.5  $\mu\text{g/g}$  in the bedding. The change in allergens in the two groups was presented in a figure (see below), and it is typical of research in this field that no formal tests of statistical significance were given:

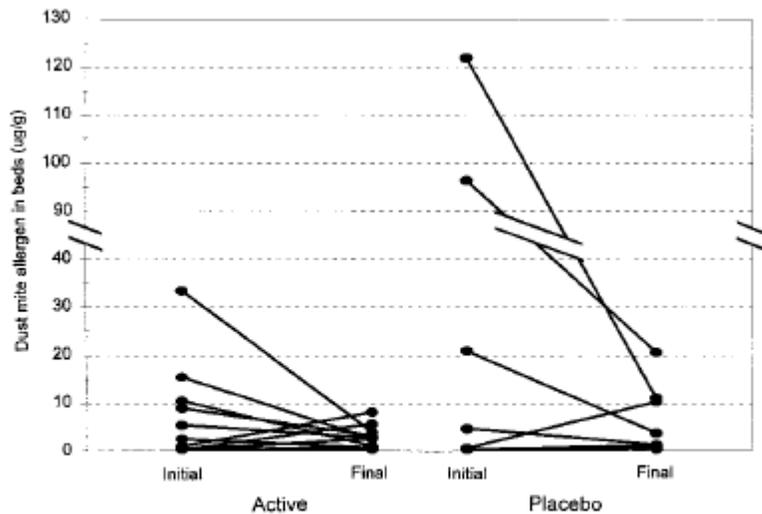


Figure 1. Change in dust mite allergen levels in bedding at initial and final visits by treatment group. The active group included 11 houses of which two had  $<0.4 \mu\text{g/g}$  at initial and final visit. The placebo group includes nine houses of which two had  $<0.8 \mu\text{g/g}$  at initial visit and follow-up.

Marks and colleagues only provided their control group with a placebo acaricide spray, yet there was a greater reduction in bedding HDM allergen in this group compared to the active group, who were given impermeable encasings and acaricide spray, which suggests that something quite beyond the usual suspects caused these changes, and the most likely culprit is natural random variation [100].

Random variation in allergen-levels is the more plausible explanation for why there was a statistically significant reduction in the Acares test in the active group after mattresses were sprayed with acaricide in Easter, yet there was not a significant reduction after they had initially been sprayed during the prior Christmas [105].

Although the majority of studies did not demonstrate a biologically plausible causal correlation between lowered allergens and improved asthma, there were two studies that did. Ehnert and colleagues found that there was relatively steady improvement in bronchial hyperactivity over the duration of the 12-month study, with only a slight decline in improvement at twelve months [94]. It is biologically plausible to argue that this was caused by allergen-reductions, because allergens reduced significantly over this same time period. Carter and colleagues also showed that there was a statistically significant correlation between allergen-reduction and improved asthma, when analysis of their results was restricted to subjects who were mite sensitive, and who therefore possessed a biologically plausible ability to respond to allergen-reductions [91]. Furthermore, when analysis was restricted in this way, there was evidence of a dose-

response relationship between the magnitude of the reduction in subjects' allergen-levels and the magnitude of the reduction in their acute-healthcare visits. This demonstrates the value of excluding subjects who are not sensitised and whose allergens did not reduce, because when analysed without this restriction Carter and colleagues' study did not show a significant correlation between allergen-reduction and improved asthma and indeed, as was mentioned above, their interventions may not be the cause of the allergen-reduction.

The research reported by Morgan and colleagues was a sentinel study because it recognised the necessity of only studying children for whom there is a biologically plausible pathway between allergen-reduction and improved respiratory health [66]. They did this by excluding from enrolment children who had a negative skin prick test, and by using multiple interventions that substantially lowered allergen levels. However, their allergen-reduction findings could have been due to natural variation rather than the interventions, and their asthma outcomes could have been due to natural variation and/or the placebo effect (as was discussed in section “6.11 Home visit±education±placebo-effect±Hawthorne effect”).

### ***2.21 Consistency of findings between studies in the review and other studies***

Visual inspection of Table 3 shows that the most consistent outcome of the trials was that there was no change (either positive or negative) in asthma; and that when asthma had changed, it had improved more often than it had deterioration. In fact, only two studies

witnessed deterioration in the active group compared to the control group. Four children in Hayden and colleagues' active group compared to two in the control group were hospitalised because of asthma (the statistical significance of this comparison was not reported) [95]. The only other study that observed a worse outcome in the active group compared to the control group, was underpowered and the results were not statistically significant [106]. Table 3 also consistently shows that the asthma outcomes that improved the most, were outcomes for which there was potentially a large psychological component (asthma symptoms, acute healthcare visits, quality of life) and may be influenced by the placebo and Pygmalion effects (see Appendix 3).

The two trials identified in the present systematic review by Boner and colleagues [105, 110] used a study design that appears to have been replicated on an annual basis, and hence it is possible to compare these studies with one published earlier in 1985 [33] and the one published later in 1994 [105]. This comparison illustrates the lack of consistency of the bronchoprovocation results: the 1985 trial showed improved exercise-induced bronchoprovocation (exercise was used in the 1985 study as a more humane way to test bronchoprovocation), but there was no improvement in histamine-induced provocation in the 1993 trial, or methacholine-induced provocation in the 1994 trial.

Williams and colleagues observed that allergen levels were lower in the active group compared to the control group at the end-of-study, but this is not consistent with other trials that reported similar differences in allergen levels between groups at study completion. Most studies which produced a reduction in the active group's allergens

compared to the control group, achieve this by lowering the active group's allergens compared to baseline. However, in the study by Williams and colleagues, the allergens did not decrease in the active group and they only reason the active group's allergens were lower than the control group was because the control group's allergen levels inexplicably rose dramatically. While that is biologically plausible to argue that the active interventions prevented the active group's allergen levels rising like how they rose in the control group, it is not consistent with the pattern in other trials, because other positive trials reduced the allergen-levels in the active group (end-of-study compared to baseline).

Table 3 illustrates the inconsistency between trials in which asthma outcome improved, out of the range of asthma outcomes that they measured. For instance, Ehnert and colleagues found an improvement in bronchoprovocation, but seven trials found no improvement; asthma symptoms scores improved in six studies, but were unchanged in an equal number of studies. The lack of consistency in allergen-reduction trials is perhaps the key reason why there is serious doubt about the allergen-reduction hypothesis.

## ***2.22 Strengths and weaknesses of the present systematic review***

The strengths of the present review were that it was conducted in a systematic manner, and it did not include studies from reference lists and thus it avoided citation bias.

The search strategy was designed to be extremely focussed and have a high specificity rather than a high sensitivity. However, this meant that it excluded a number of allergen-reduction trials. There appear to be inexplicable omissions from the review, for example the search strategy identified the 1993 paper by Boner and colleagues but did not pick up the 1985 paper by the same group of authors. In addition it flagged the Inner City Asthma Study by Morgan and colleagues, but did not detect some of the other groups who published very similar trials to the Inner City Asthma Study such as [82, 148]. Interestingly, the search detected the preliminary report by Krieger and colleagues that described their study design [99], but it did not detect the subsequent follow-up report that contained the results of this study [98]. These omissions are unlikely to have biased the study, because the search strategy was not biased, and because it was applied systematically. The omissions are testament to the quirks of Ovid, and the vastness of the allergen-reduction literature.

In recognition of the limitations of the present systematic review, the results of published systematic reviews have been mentioned throughout the present review, and are also presented in Section 7 below. The majority of the published reviews supported the conclusions of the present review.

## **2.23 Conclusions**

The present systematic review has unveiled a number of shortcomings in the designs of many of the published allergen reduction trials, the two most prominent of which were,

the absence of a control group, and the inability to take account of the placebo effect in trials that did have a control group. Many of the trials found that asthma improved with allergen-reduction, and although their methodological flaws prevent their findings from being conclusive, they do justify the conduct of more rigorously designed trials to test the allergen-reduction hypothesis.

### **3 What published systematic reviews have found**

Systematic reviews have been conducted of various single interventions, such as air filters [85, 87, 149], air ionizers [150], humidity control [151], allergen-impermeable bedding encasings [152], educational interventions [153], the role of lay health workers in the community [154], feather versus non-feather duvets [155], smoking control [156, 157], and individualised written action plans [158]. Systematic reviews of multiple interventions have also been published, such as multifaceted interventions for indoor air modification [159]. Other systematic reviews focussed on particular allergens, such as pet allergens [85] and HDM allergens [86, 160]

The majority of the published systematic reviews were consistent with the review conducted as part of the present thesis, and supported its conclusions that many trials had flawed study designs, there was no conclusive evidence to support the allergen-reduction hypothesis, and that the interventions that did improve asthma were likely to have worked via a psychological and educational mechanisms.

The absence of improvement in a number of asthma outcomes in many of the trials in the present review (Section 6) are consistent with systematic reviews, which found no effect of humidity control [151], and no effect of counselling parents not to smoke around their asthmatic children [156]. Likewise, most reviewers concluded that air filtration was ineffective. Fox wrote a non-systematic review of air cleaners and concluded that air cleaners did not lower allergen levels [161]. McDonald and colleagues reviewed air

filters and found that five trials had tested air filters in asthmatic children and there was a statistically significant lower total symptom score and lower sleep disturbance; but the small sample size and heterogeneity in the results, reduced the likelihood that this represented a causal association [87]. Kilburn and colleagues also concluded that there was no effect of air filters for reduction of pet allergen to improve asthma [85].

Not all the reviews concluded that there was no evidence of improved health from allergen-reduction interventions. Two reviews judged that the interventions they reviewed had improved asthma: a review of interventions led by lay health workers found good evidence for their effectiveness compared to usual care [154]; and a review of written action plans (particularly those focused on symptoms rather than just peak flow) improved children's asthma [158]. It is likely that much of the improvement in asthma from allergen-reduction trials comes from their use of lay health workers and written action plans. These two positive reviews cannot rule out the possibility that the Hawthorne and Pygmalion Effects, rather than allergen reduction, caused the improvements in asthma.

The present review identified the impossibility of drawing any firm conclusions from many of the trials because of defects in their study designs and the way they were reported, which was consistent with the conclusions of other systematic reviews. For example, Campbell and Gibson noted that none of the studies of feather-duvets versus non-feather-duvets could be included in their Cochrane review because the studies in the literature had so many methodological flaws, such as a lack of randomisation [155].

However, these reviewers could be criticised for being overly purist, because they only included trials that investigated feather versus non-feather duvets as the sole intervention; and hence they excluded all multi-intervention trials that used feather versus non-feather duvets plus other interventions.

Custovic and colleagues also commented on deficits in trials' study designs, and in particular on the lack of a control group in many of the earlier studies [162]. Interestingly, Custovic and colleagues warn that, although ecological studies give clear evidence of an association between allergens in the indoor environment and the prevalence and severity of asthma, there is no definitive evidence from intervention studies, that a reduction in these allergens will cause an improvement in symptoms of asthma [162]. Other reviewers have also pointed out the lack of a control group in the early trials of allergen-reduction, and the inability to have a genuinely blind study due to the absence of a matching placebo intervention [151].

Gøtzsche and colleagues noted that because many studies were poorly designed, they may inflate the efficacy of the interventions [163]. Despite this risk, a meta-analysis of the trials showed no statistically significant differences in the relative risk of asthma outcomes in active compared to control groups [164].

Custovic and colleagues criticized the systematic review of Gøtzsche and colleagues for concluding that there was insufficient evidence to recommend allergen-reduction methods, because only four of the 23 studies included in their review actually reduced

allergen-levels and those four did show that asthma improved as allergen levels declined [162]. However, what Custovic and colleagues failed to recognise is that if the interventions only had a 4/23 (17%) success rate, this may not be enough to justify their expense. Indeed, in Custovic and colleagues' own review, only nine out of 31 trials actually reduced allergen levels, and although these nine studies showed that some markers of asthma improved, other asthma outcomes did not improve. Custovic and colleagues appear to be suggesting that researchers should abandon an intention-to-treat analysis, and restrict the analysis to only those subjects who did experience reduction in their allergen levels, however this can introduce bias and confounding, and should only be done with caution.

Most reviewers judged that there was modest evidence that allergen-impermeable bedding encasings do improve asthma. Recer reviewed over 30 clinical trials of encasings, and concluded that fourteen trials reduced allergens, of which four led to reduced BHR, but in ten trials there were no statistically significant reduction in BHR in the active compared to control groups [152]. Interestingly, a sub-analysis of subjects who had had high HDM allergen levels at baseline ( $>2\mu\text{g}$ ), showed that there was a modest correlation between allergen reduction and BHR, which is consistent with the interpretation in the present review that the baseline levels were just too small in many of the trials for any reduction to have a noticeable immunologic effect.

## 4 A critical review of the present and published reviews

The conclusions of the systematic review conducted as part of this thesis, were consistent with those published in the literature. The key findings of these reviews were:

1. there were methodological challenges posed by conducting trials in subjects' homes;
2. there were ethical and practical difficulties which limited the development of placebo interventions;
3. most studies were unable to determine whether any improvement in asthma was caused by allergen-reduction, or by the positive psychological effect of taking part in research.

Trials need to have external validity, in order for them to have any practical application, and therefore allergen-reduction trials must be conducted in children's homes. However, the nature of children's homes and family life prevents some interventions from being fully deployed, and some outcomes measures from being objectively observed; which limits the internal validity of these trials.

For a trial to have internal validity, it must have a genuine control group, which is masked and to which the allocation is randomised. The control group allows the trial to take account of the natural history of asthma, and psychological processes. However, because these trials are conducted in families' homes, the interventions are highly visible to the subjects, and are often carried out by the subjects themselves, which makes it

difficult to create a genuine control group, because it is not easy to mask subjects to their assignment.

Psychological processes have a powerful effect on both subjective and objective asthma outcomes. The psychological impact of asthma education and allergen-reduction education is effective at improving asthma [153, 165, 166]. The effect of education is so powerful, that it may be a necessary component to all asthma intervention trials [91, 98, 112], particularly to encourage compliance [167].

In order for trials to attribute any improvement in asthma to the effects of allergen-reduction, they must ensure that the control group experiences the same psychological benefits as the active group by giving the control group a placebo. However, the use of a placebo may obscure any beneficial effect of allergen-reduction in the active group. A placebo must outwardly appear to be a genuine intervention, but this cannot be done without also making the placebo somewhat effective at lowering allergens or improving asthma. Ideally a placebo would not have any positive effect on asthma, however, it is not ethical to give the control group a treatment that is known to be less effective than standard medical therapy [168]. Large sample sizes are required in allergen-reduction trials, because it is inevitable that a placebo will improve asthma outcomes, and reduce the size of the difference in improvement in asthma in the active versus control groups.

Most studies in this review either did not use a placebo at all, or used a placebo that would not have been very convincing, and therefore would not have had a placebo-effect.

This point was also recognised by Francis and colleagues who acknowledged that their lack of a placebo air filter for their control group meant they could not rule the placebo effect [169]. Indeed, the only published placebo-controlled trials of interventions to modulate allergy, are ones that test pharmacotherapies for which placebos of identical appearance can readily be manufactured [170-174].

Most of the trials in this review were not double-blind trials, because they did not have placebos that looked identical to the active interventions. This could have led to differential treatment of subjects in the two groups by researchers who delivered the interventions, and researchers who collected the outcome data. Some researchers tried to mask the collection of outcome data, by doing as much of it as possible over the telephone by separate staff, who were blind to subjects' allocation (so they could not see what interventions were present). However, during such phone calls, it would not have been long before the subjects mentioned something about the trial that made it clear which assignment they had been allocated to.

Not only were there practical barriers to the design of placebo interventions, there were also practical limitations to the design of ideal active interventions. Few studies achieved large reductions in allergen levels, and no study lowered them from being above the threshold for causing symptoms at baseline, to being below the threshold throughout the entire duration of the study. It is very difficult to create environmental conditions that kill mites. For example, even with intensive MVHR, humidity levels never dropped

below 50%, but mites only die if levels are below 40% to 45% [175], which explains the minimal reduction in Derp1 and modest clinical results of Warner and colleagues [107].

Several instances of multiple publications were noted in the present review, which may give the appearance of a greater body of evidence to support allergen-reduction than what actually exists. While it is advantageous that research findings are widely disseminated in a variety of media, multiple publications can result in misconceptions [176-179]. Intriguingly, a study of allergen variability in carpets was published twice in *Indoor Air* within a few issues of each other, the only difference being in the size of their tables [145, 146].

These systematic reviews show that it is necessary to test the allergen-reduction hypothesis with a double-blind randomised placebo-controlled trial. For this reason, we designed a trial as close to this ideal as possible (we could not double-blind it); and piloted its acceptability to asthmatic children and their families.

## **5 The Pilot Study**

The Health Research Council funded a team of researchers, led by the Principal Investigator, Professor Julian Crane, to design a pilot study, based on the trial of Morgan and colleague, with the addition of placebo interventions for the control group, and then to pilot its acceptability among New Zealand families. Ethical approval was gained from the Central Ethics Committee. Brent Caldwell; Cheryl Davies; and Professors Crane, Howden-Chapman, and Cunningham, had several meetings to design the trial; and Brent conducted it with the help of Rangi Eria.

### ***5.1 Adaptation of the American study design***

The pilot study was based on the study by Morgan and colleagues [66], because it was one of the few trials that produced both, a durable reduction in allergens, and a statistically significant improvements in children's asthma. The hypothesis of the pilot study was that the American study could be adapted so that it had placebo interventions, and that it would be acceptable to New Zealand families. Morgan and colleagues used multiple interventions against a wide range of allergens and irritants, asthma education, and psychological support, and found that it caused modest but statistically significant improvements in asthma (see Tables 2 and 3).

### **5.1.1 Relevant differences between USA and NZ**

It was necessary to adapt the American study design because of several important differences between New Zealand and the United States of America: the climate; the type of housing; the social environment; cultural issues; and economics.

New Zealand has a much more humid climate than in Tucson, Arizona (In December 69-74% humidity in Wellington and 34-62% in Tucson [180, 181]), so opening windows and improving ventilation is not necessarily going to decrease indoor humidity in New Zealand.

In New Zealand there is not the same level of violence and social problems as the United States, and hence we hypothesised that it would be unnecessary to undertake the social worker counselling of families. Because we were not planning to provide social worker support, it was not ethical for us to ask many of the very sensitive personal questions that had been used in the American study, such as questions about abortions and family violence, because we would have been unable to respond with the appropriate level of support if these questions were distressing or it became apparent that some help was required. It is also not as culturally appropriate in New Zealand to ask questions about sensitive issues such as abortions. However, we may have underestimated the extent to which this intense personal contact and support of subjects by the research staff, is required to ensure that subjects complied with carrying out the interventions and recording their asthma outcomes.

It was important to make certain that the wording of questionnaires, and the ‘script’ for explaining how to carry out the allergen-reduction interventions, were culturally appropriate for Māori.

New Zealand families are not necessarily wealthy enough to pay for the extra electricity that would be required to use hot enough laundry water to kill mites as was done in the American study, so we used a Eucalyptus oil emulsion instead, which subjects soaked their laundry in before washing it. Eucalyptus oil is acaricidal [182].

### **5.1.2 What to use as a placebo?**

There are many allergen-reduction interventions for which it is impossible to have a placebo with identical appearance, such as modifications for the structure of the building to stop leaks or to remove surfaces ingrained with mould [96].

We decided that, wherever possible, we would have placebos that outwardly appeared to be identical to the active interventions, but that where this was not possible, or not desirable, we would use as a placebo something that could provide a placebo effect. This had the advantage of accounting for the placebo effect, but it did detract from our ability to blind the subjects and researchers because both groups were not receiving identical interventions. This was particularly an issue for the bedding encasings, for which we provided dehumidifiers as the placebo equivalent. We felt that in order to remove the

placebo effect, it was not necessary for each intervention in the control group to have a matching active intervention in the active group, but that it was more important that the placebo interventions *collectively* were as convincing and plausible as the active interventions. However, this did mean that subjects would not be blind if they talked to subjects in the other group, and researchers were not blind.

## **5.2 Method**

Single-blind, randomised, placebo-controlled trial design.

### **5.2.1 Study setting**

Homes of families living in Wellington and the Hutt Valley, who used the services of Tu Kotahi Māori Asthma Trust.

### **5.2.2 Subjects**

We aimed to enrol ten subjects. Subjects were recruited by Rangi Eria, a community worker who works with families as part of the Tu Kotahi Māori Asthma Trust service, and whose cultural guidance was essential to the conduct of this trial.

There were three stages in the process of assessing whether subjects met the inclusion/exclusion criteria. The first set of criteria were: *Inclusion criteria*: children aged 5-11 years old, with physician-diagnosed asthma, whose parents had a low income; who spent at least five nights of the week in one house; and who had at least one hospital admission or two unscheduled visits to the GP, A+E, or other acute health provider due to asthma during the past six months. Subjects were delayed from entering the study, until at least three weeks had past since their last exacerbation. *Exclusion criteria*: oral corticosteroids use; other significant respiratory disease; any serious chronic illness.

Subjects who met these initial criteria then had to have a positive SPT to one of the allergens in the study: HDM, cat, dog, moulds, fungi, grass, and tree pollens. Subjects who were not atopic were excluded at this point.

Subjects who had the required SPT results were then visited in their homes to determine if the allergens were present in their home environment. If the source of the allergen that they are sensitised to (such as mould or cockroaches) were not visible in their home then they were not enrolled in the study.

### **5.2.3 Interventions**

Active group (six children): All children received allergen impermeable mattress, duvet, pillow, bed-base covers. Caregivers were advised to not allow anyone, including guests, to smoke inside. All households received a HEPA filter vacuum cleaner and instructions

for children to stay outside the house while the vacuum cleaner was being used and for 30 minutes afterwards.

*Dust mite module:* For a child that had a positive HDM SPT, their family was advised to remove carpet in the child's bedroom if they could, and advised to vacuum and dust the child's bedroom daily if they could. Eucalyptus oil emulsion was provided to add to all laundry washing. We encouraged weekly washing of bed-sheets, and for all laundered sheets and clothes to be dried outside in sunlight.

*Passive smoking module:* If a member of the household smoked, then the author educated smokers about the effects of passive smoking and counselled them to smoke outside. Smokers were offered a referral to the publicly-funded Quitline.

*Cockroach module:* If the child had had a positive SPT and there was evidence of cockroaches inside, then, a social learning theory-based approach was used to educate parents about ways to make the home less favourable for cockroaches, and we also offered a professional exterminator service.

*Pet module:* If positive SPT and the corresponding pet was present (or had been present in the past two weeks), we then suggested (by providing a good explanation of the benefits using social learning theory) that the pet be kept outdoors, and if that is unfeasible, we then suggested that it not be allowed in the child's bedroom.

*Rodent module:* If there was a positive SPT and evidence of rodents, then we educated the parents about rodent avoidance, and offered a professional exterminator.

*Mould module:* If there was a positive SPT and if mould was present then we demonstrated how to remove the mould with a vinegar solution, and educated the household, using social learning-theory, about mould and damp avoidance, for example

drying clothes outside. A housing inspector would also inspect the house and advise on the structural aspects of the house that create favourable conditions for mould growth using a standardised Healthy Housing Index.

Control group: (four children): We saw no need to pilot the passive smoking or mould modules, because they only involved giving advice and a pamphlet. Caregivers were advised to not allow anyone to smoke inside. All households received a vacuum cleaner that looked identical to the one provided to the active group (but it had a non-HEPA filter inserted where the HEPA filter would normally have been), and instructions for children to stay outside the house during, and for 30 minutes after, use of the vacuum cleaner.

*HDM module:* if the SPT was positive then we provided equipment and instructed parents to wash bed-sheets and all clothes weekly, and to add placebo acaricide to the laundry water. We gave no advice about how to dry laundered sheets and clothing, and let subject use whatever method they preferred. We provided dehumidifiers, and instructions for them to be placed in the child's bedroom and turned on for one hour a day.

*Cockroach module:* if the child had a positive SPT and evidence of cockroaches in home, then we provided a written leaflet on cockroach avoidance, and arranged for a sham cockroach extermination.

*Pet module:* if positive SPT and the corresponding pet were present, then we advised that the pet not be allowed in the child's bedroom.

*Rodent module:* if positive SPT and evidence of rodents, then we provided leaflets about avoiding rodents, and provided a sham extermination.

#### **5.2.4 Data collection**

**Outcome measures:** The primary outcome was the acceptability and practicability of conducting the study. This was assessed by listening to participants' comments, and observation made by the author. PEFR and Juniper Paediatric Asthma Quality of Life Questionnaire were recorded by parents for a fortnight every two months on forms that were left with the parents when the interventions were delivered. No dust samples were collected in the pilot. At the end of the study, a questionnaire was administered to gain a quantitative outcome measure of the acceptability of the study.

#### **5.2.5 Analysis plan**

Two types of data were collected. Quantitative data were collected on the range of SPT sensitivities and allergens present in the homes, and the questionnaires and diaries that participants completed. The other type of data were the qualitative impressions that the author formed of the process of carrying out the study and what the participants' experience had been of taking part in the trial. The conversations were not recorded or transcribed, and no formal method was used to record or analyse these qualitative data, which were formed by the author's subjective impressions from spending time with the subjects.

## **5.3 Results of the Pilot study**

### **5.3.1 Subjects**

Subjects were identified through searching the patient lists of Tu Kotahi Māori Asthma Trust, and discharge summaries of patients admitted to Wellington Hospital Paediatric Ward. Seventeen children were formally screened, of whom seven did not meet the inclusion/exclusion criteria. Of these seven children, three had either not been hospitalised, or not had two unscheduled GP visits for asthma in the previous six months, and four were found to not meet the skin prick test requirements (three were non-atopic and one was only sensitised to grass pollen).

### **5.3.2 Skin Prick Test results for enrolled subjects (plus one sibling)**

The prevalence of skin prick test reactions for the ten subjects (plus the brother of one of the subjects) is provided in Table 5. As mentioned above, four of the seventeen children who were screened did not have the appropriate atopy.

### **5.3.3 Allergen Exposure**

The allergen exposure of children was quite varied and most children were exposed to multiple allergens (see Table 6). An allergen was considered to be present, if it was either seen during the home inspection, or if the caregiver reported that it was or had been present.

### **5.3.4 Acceptability / practicality from the point of view of the researchers**

#### **5.3.4.1 Incomplete questionnaires and symptoms diaries**

Not all parents recorded their children's asthma symptoms and outcomes, and those who did record them, did not always record all the outcomes. Commonly parents would record either the morning or evening peak flow, instead of both. Part-way through the trial, a little picture of a peak flow meter was added to the two parts of the diary chart that record morning and evening peak flows, to try and make it clearer that they both need to be answered.

The failure of parents to record outcomes thoroughly, may be an indication that the explanation that was given to them about the value of regular monitoring of asthma (in

terms of improved asthma control) may not have been appropriate, and the technique for explaining this needs to be improved. In addition to missing peak flows, parents usually did not circle 'no' to indicate that a symptom was absent – they would just indicate absence by leaving the 'yes' uncircled. It was explained to parents that the absence of a circle around 'yes' could just mean that they forgot to answer the question and did not necessarily mean that their child had not experience that symptom.

#### **5.3.4.2 Difficulty scheduling appointments for home visits**

One subject spent most of his time living in his mother's house (who enrolled him in the study), but also spent quite a lot of time living at his father's house (his parents are separated), which made it difficult to schedule visits. Children from split families may need to be excluded from this study.

One mother was unable to be at home for the first home visit, and had arranged for her boarder to be at home instead. So the researcher had to give all the education about the interventions to the boarder instead of to the mother. At the next visit it became clear to the researcher that the boarder had not given the mother a detailed description of how to use the products. It is important for researchers to reschedule appointments to make sure that the main caregiver receives the education, not someone who is just standing in for them.

The author had to spend a substantial amount of time to reschedule visits to the homes of five of the subjects, who missed appointments. Three of those five subjects only missed one appointment, whereas one parent missed three appointments. It was this author's impression that most of the failure of subjects to be home at the time of the appointments, was not due to a lack of interest in the study, but rather it was due to the subjects having significant work commitments and having jobs which required them to work extra hours at short notice. A further reason was the parents had wider family commitments that they could not always anticipate (such as the need to take an extended family member to work because her car had broken down). For the national study these issues could be overcome by:

- providing subjects with written appointment cards (instead of only making the appointment verbally);
- ensuring researchers have enough time to phone subjects a day or two before the visit to remind them of it (although one subject failed to make an appointment she was reminded of the day before);
- making sure that prospective subjects are made fully aware that participation in the trial will require a lot of their time on an ongoing basis, and are not encouraged to join the study simply so that they can get free vacuum cleaners etc;
- visiting the subjects a few days after the interventions are delivered, to reiterate how to use the interventions, and to quickly identify if subjects need further education and encouragement;
- having space on the questionnaires, to keep track of all the times subjects were phoned, to help researchers keep track of all the issues that subjects have had, and

all the times their appointments have been rescheduled, and why appointments were rescheduled.

One father (who had been at work when the interventions were delivered to his wife) repeatedly phoned to cancel the home visits, even though they had already been rescheduled to a time that suited the family. The researcher formed the opinion that this father was very domineering, and that his wife and daughter were quite intimidated by him. Clearly, conducting home interventions and visits, exposes very sensitive issues, such as potential emotional abuse within families, which can impact on the children's asthma, and may also raises ethical obligations for the researcher to report abuse to child protection agencies.

When it was too complicated to arrange a final home visit, the researcher posted stamped self-addressed envelopes addressed to the subjects for them to return their completed questionnaires/diary-cards. However only one of three subjects who were sent this pre-paid envelope used it; the other two subjects did not return the envelope or the questionnaires. It is essential to go and visit the house to pick up questionnaires, not get them posted back.

#### **5.3.4.3 Visits were too demanding on parents**

Part of the study protocol is to demonstrate the interventions to the families, and use Appreciative Inquiry [183] to encourage the new behaviour. However, several parents

were reluctant to remove their child's bedsheets to allow the researcher to demonstrate how to use the acaricide. Parents were reluctant to do this because they were far too pressed for time, and they may also not have seen the point of being shown how to do what might appear to be an easy routing household chore.

#### **5.3.4.4 Visits were too demanding on the researcher**

Some aspects of the home visits were very taxing on the researcher. For instance it was very demoralising to turn up to a subject's home only to find that no-one was home, and then have to wait to see if the subject was just running late. Trying to make contact with parents, to reschedule visits was very time consuming.

The dehumidifiers were very heavy, and it was difficult to lift them out of the car and up the flights of steps into subjects' homes.

#### **5.3.4.5 Subjects needed reminders**

One subject suggested that we should provide a sticker/poster to put in the laundry-room to remind parents to put the acaricide into the washing machine, because the acaricide must be stored away from sight (so children do not drink it) and so it can easily be forgotten. Another parent said that it would be helpful if the acaricide bottle was labelled

with the instructions on how to use it, because she had forgotten how to use it and had forgotten where she had put the instructions.

#### **5.3.4.6 Difficult to contact some subjects**

It was difficult to contact some subjects, particularly if they did not want to give us their daytime work phone numbers because they were not allowed to receive personal phone calls during work hours. Often these subjects did not have answerphones on their home telephone numbers, so it was impossible to contact them unless researchers phoned outside of normal work hours. It is important to have sufficient funding to employ enough research staff to take turns at working after normal business hours, to accommodate the needs of subjects. It would have been useful if we had made a note of what time of the day would suit subjects to receive phone calls, and which phone number to use at that time.

#### **5.3.4.7 Difficult to find appropriate pest control professionals**

It was not straightforward to find appropriate pest control services. Ideally in the main study a pest control company would be employed that is able to supply the same service in all study centres – Wellington, Christchurch, and Auckland. The national companies, such as Rentokil, are not staffed by people who understand the science behind what they do, and are only able to follow inflexible company procedures, whereas some of the sole-

traders are well educated about pest behaviour and are flexible in what they can do (for instance, they are able to use placebo bait). However, the majority of the sole traders that the author contacted were difficult to talk to, and did not supply quotes despite requests for them.

#### **5.3.4.8 Very expensive interventions**

Quotes for pest control varied from \$150 to \$220 per pest, which is very expensive considering the number of pests that must be controlled: mice, rats, German cockroach, Asian cockroach, Oriental cockroach, American cockroach and mould.

Professional building inspections are very expensive and cost \$500 to \$1,000 per house. In the pilot study, a building inspection was not undertaken.

Manufacturing the Eucalyptus oil emulsion will be very expensive, because the ideal emulsifying agents, like Tween-20, are expensive; and the homogeniser to mix the oil and Tween-20 is extremely expensive. We did not use a homogeniser, and as a result, our emulsion tended to split and separate out into a liquid layer and a separate oil layer, instead of remaining as a homogenous emulsion.

### **5.3.4.9 Some allergen exposures could not be controlled by the interventions**

One parent reported that her son's recent asthma exacerbation occurred after he had been rolling around with his friend in long grass (his SPT was positive for grass), however it is probably not possible to stop children from playing in grass just because they are allergic to it.

### **5.3.4.10 Subjects can break the intervention equipment**

One dehumidifier broke, and it is possible that it broke when it was dropped by the subject, because he was seen carrying it even though it was almost as big as he was. It would be very difficult for parents to stop their children from playing with the interventions, and so the cost of replacing broken interventions must be factored into the costing of research.

### **5.3.4.11 Subjects lose the interventions and/or questionnaires/diaries**

One child lost one of her symptom diaries – her mother also lost the acaricide when they moved house. One other subject lost her symptom diary when she took it to school for “show and tell”.

#### **5.3.4.12 Discordant approaches between parents**

Not all parents / caregivers agree with each other about the nature of their child's asthma, or how it should be treated. This lack of unity can compromise parent's compliance with the intervention protocols. For example, fathers were rarely at home when the interventions were delivered to the mother, because this usually happened during normal working hours, and therefore the fathers did not get a chance to receive the education from the researchers and therefore some fathers were very sceptical about the study and discouraged the mothers from sticking to the study protocol.

#### **5.3.4.13 Fairness and sibling rivalry**

One family in the pilot study had two children who met the inclusion/exclusion criteria for the study. In order to be equitable, and to avoid family strife, we provided both children with the interventions, however this doubled the cost of the study. The siblings must be randomised as a block, so that they both get assigned to the same group (unless the interventions in the two groups are absolutely identical in their outward appearance). In a similar vein, in a different family in the study, the asthmatic child shared a room with her non-asthmatic sibling, and sometimes sleeps in her sibling's bed. We would need to provide the sibling with the same allergen impermeable bedding encasings.

#### **5.3.4.14 Exclude children who already have one of the interventions**

One child already had allergen impermeable covers on his bed, which his parents had purchased because they had heard that the covers were good for asthma (even though they did not know if their son was HDM sensitive). If this subject had been randomised to the active group, then this would not have mattered because the study encasing could be put over the top of the one that the family had bought, but if the boy had been randomised to the placebo group, then the family's encasing would have had to be removed, which is not fair. It would be more straightforward to exclude children who already have one of the interventions in their home, however this increases the number of exclusion criteria and makes it even harder to recruit participants.

#### **5.3.4.15 Community workers are untrained in research methodology**

One of the community workers who assisted with recruiting families to join the study, said that she had encouraged people to enrol in the study because it will provide them with free vacuum cleaners, free dehumidifiers and/or free mattress encasings. It is possible that by emphasising these free interventions and perhaps not pointing out the purpose of the trial and the considerable work-load that it will impose on them (conducting the intervention procedures in their homes, recording symptoms etc) people may have been motivated to enrol to get the free interventions and they may not have had

a firm resolve to follow the study protocol in terms of recording their data and being at home at the time when the researcher was scheduled to visit them.

### **5.3.5 Acceptability / Practicality from the point of view of the subjects**

#### **5.3.5.1 Some interventions were incompatible with subjects' lives**

One parent wanted to cover the mattress encasing with a plastic sheet and then cover that sheet with a thick blanket, because her son had nocturnal enuresis. Without the plastic sheet the mattress would get soaked with urine. However, this thick blanket would harbour HDM and defeat the purpose of the encasing. Yet, it was uncomfortable for the child to sleep directly on top of the plastic sheet.

Mattress encasings do not allow for the use of an electric blanket, which meant that the electric blankets had to be removed from one of the beds in the pilot study. While this is not too much of a problem in relatively warm houses, it could be an issue in colder homes.

### **5.3.5.2 Some interventions and outcome recording was too demanding**

One mother said that she thought most parents would get pretty hoha<sup>IV</sup> with having to record their children's symptoms every day for each two week period. This might be due to the fact that she had to record them for both her boys, and if she had had to record only one child's symptoms she might not have found it to be such a nuisance.

About half of the mothers said that their main problem with the study, was the requirement to wait for 30 minutes for the eucalyptus oil to soak in the washing machine. If they went out (to go shopping for instance), they would come home only to find that their washing machine had not finished the washing cycle, because it was paused on the soak part of the cycle. So instead of coming home to a completed load of washing that could be hung up to dry, they came home to find that the washing had to be drained, laundry powder inserted, washed, and spun, before it was ready to be hung up. Several mothers said that they had a lot on their minds, and would do their washing with their minds on automatic pilot, and so they would forget to pause the machine, put the acaricide in, and let it soak; or if they remembered to let it soak, they would forget to start the cycle again after the soak was over. This comment was made by mothers regardless of their level of education, or financial resources, and whether they had full time jobs or were unemployed.

### **5.3.5.3 Dehumidifiers had a powerful placebo effect**

Several parents mentioned that they thought the dehumidifiers were very effective (possibly due to intensive marketing of them on television). One mother was extremely happy at the end of the study because she firmly believed that the dehumidifier was extremely effective and had made a big difference to their symptoms. She put the dehumidifier in the bedroom that her two asthmatic sons slept in, and kept the dehumidifier on all night, and said that it did not disturb her sons' sleep. However this was longer than the protocol required, and would have increased her electricity bill considerably.

## **5.4 *Conclusions from the pilot study***

The pilot study confirmed many of the conclusions that other researchers had reported in the literature. In particular, it confirmed that allergen-reduction interventions impose a substantial burden on subjects, who require considerable input (education and resources) from community workers, to give subjects the motivation and resources to fully participate in the trial. The pilot showed that study implementation is taxing, and in the main study it would be necessary to employ a large number of research staff who are able to work flexible hours outside of normal office hours to enable them to fit in with the often chaotic lives of subjects, and to visit subjects at times when all the relevant family members are present, to ensure that all family members understand and support the trial,

and can work in harmony to undertake the interventions and recordings. It showed that families were interested in taking part in the research, and that if the researchers were able to spend more time, more often, with subjects, then their interest in the study and their compliance with its protocols is likely to be maintained. There are some aspects that may need to be modified (such as posters to remind subjects of procedures, and a way to make the placebo interventions look identical to the active ones). With some modification of the interventions, this study design is likely to work well, and lead to useful, interpretable conclusions.

## 6 Discussion of the Review and Pilot Study

This Chapter (Chapter Six) weaves together the lessons gleaned from the present systematic review, with those learned from the pilot study. In Chapter Seven, those lessons are applied to the design of an ideal study protocol that would be capable of testing the allergen-reduction hypothesis.

Medical knowledge of immunology and asthma strongly suggests that a reduction in allergens should improve asthma. However, because the mechanisms of allergy and asthma are extremely complicated and not fully understood, our current hypotheses must be treated with caution. This is especially so for the allergen-reduction hypothesis, because allergen-reduction interventions require enormous resources (time and money), and they can have unexpected adverse effects, such as increased night-time cough [106].

Yet to test this hypothesis requires very complex study designs in order to overcome the practical difficulties of conducting trials in the domestic environment. These difficulties often lead to the use of non-ideal study designs, which can make them difficult to interpret.

The key problems with drawing conclusions from studies in the literature relate to:

1. low levels of allergen at baseline, so there is little room for improvement;
2. several studies found allergens reduced in both the active and control groups, so the control group did not act as a true ‘negative control’;

3. inability to reduce allergens to low enough levels. (But there is uncertainty about the threshold allergen-levels for provocation. There is substantial inter-individual variability in the minimum allergen dose that provokes symptoms, and some asthmatics react to extremely low doses);
4. difficulty reducing allergens in all places that children may be exposed (all parts of the bed; carpet and fabric (sofas, curtains, clothes) in all rooms; outdoor environment (pollens, dusts, pollution); school environment);
5. difficulty in measuring the allergen that children inhale (the correlation between allergen in carpet dust and airborne allergen is not a one-to-one correlation). It is only biologically plausible that a reduction in inhaled allergens would improve asthma, yet for practical reasons, allergen levels in carpets are usually measured as a proxy for the allergen that is inhaled.
6. absence of a control group, and/or absence of a placebo intervention for the control group

Proponents of the allergen-reduction hypothesis often dispute the conclusions of trials that found no evidence to support this hypothesis, on the basis that the reason those trials found no improvement in asthma was that they did not actually reduce allergens. For example, Marks and colleagues refer to four trials that support the hypothesis and which did reduce allergens, and six trials that found no improvement, but did not reduce allergens [100]. This highlights the need to conduct systematic reviews, which take account of the totality of the research that is available; and the variability in the quality of these studies and the reliability of their conclusions. However, the present review has

detected evidence of publication bias (such as, the failure of Carter et al to report all clinical outcomes [91]), which means that systematic reviews will be biased towards missing out negative results and overemphasising positive results.

Trials that only test one intervention to lower allergens, or that do not include certain key interventions, are of no value because they are bound to support the null hypothesis. There are some vital interventions which must not be left out of studies: 1) interventions that create a safe sleeping zone, since children spend so much time in bed; 2) interventions to reduce airborne allergen, such as HEPA filter vacuum cleaners, and HEPA air filters that direct a stream of allergen-free air around the sleeping zone of beds (unlike commercially available filters) [184], 3) sufficient contact time with research staff to develop rapport and educate and involve the whole family. Future research must test multi-faceted interventions: multiple interventions per allergen to ensure that allergen is thoroughly removed; and multiple interventions against multiple allergens so all allergens that the children are sensitized to are reduced.

Advancement of knowledge about the potential for allergen-reduction to improve asthma is hampered by the fact that trials that have negative results tend to not get published, but trials that have positive results are published in more than just one publication. These problems could be improved if systematic reviewers searched trial registries, to detect trials that were registered but never reported, and if journals agreed to waive their conventional word-limits to allow all aspects of a trial to be reported in one article (so

then Carswell and colleagues would have been able to incorporate the Weeks and colleagues [103] publication in one of their other reports of this trial.

There are a myriad of complications that arise from conducting a trial in the community setting. Community workers have the interests of their community members close to their hearts, and are more highly motivated by a desire to improve their community's well-being, than by a desire to ensure that research is carried out in a scientifically robust manner. In our pilot it was noticed that the community workers would encourage people to join the study by telling them that it would provide them with free vacuum cleaners. Failure of community workers to stick to the inclusion criteria because of their eagerness to enrol as many people as possible in order that they gain free things from the study, may explain why so few of the subjects who were assessed as eligible by community workers, were actually subsequently enrolled in a trial in Atlanta [112] (another potential reason was that subjects were recruited in hospital emergency departments when parents would have been anxious and keen to participate, but once their child got better their eagerness may have waned).

It is a challenge to maintain subjects' interest in the study, and there are a number of ways to encourage this, such as via incentive payments [83, 185-187], and development of a rapport and attachment between researcher and subject [188, 189]. Factors which were associated with poor retention of subjects in a large American community trial included, parent's work schedules, problems with transport; whereas factors associated with greater retention were, group sessions at night and weekends, and incentive

payments [83]. It is not clear whether the lack of an incentive, or the failure to visit subject's homes after 5 p.m. was the reason why some subjects were hard to contact in the pilot study.

Perhaps allergen-reduction can only improve asthma early after it first develops in a child, before the child's airways are irreversibly remodelled from chronic inflammation from prolonged exposure to the inflammatory effects of allergen inhalation [63]. If allergen-reduction is instituted early after asthma is first diagnosed, and if low levels of allergens are maintained long-term, then perhaps the airway remodelling can be avoided, and asthma severity can be minimised. In fact, it turns out that there is evidence that allergen-reduction is effective in preventing sensitisation, and the development of asthma (primary prevention). A recent Cochrane review concluded that multifaceted inhaled and food allergen reduction was effective in primary prevention of asthma [190].

This section has discussed the shortcomings and challenges inherent in trials that test the allergen-reduction hypothesis in the domestic environment. The following section, outlines the features of a study protocol that would enable it to overcome these challenges.

## 7 Study Design Requirements for Future Research

This thesis ends by making a contribution toward the conduct of future research by setting out the features that future studies would have to have in order to be capable of producing meaningful results. Experts have pointed out that if research continues to use the same paradigm and structure that has previously been used, then it will not provide any further answers beyond what is already known [86]. However, these experts have not offered an alternative construct to the old ones, other than to say that future studies should “be methodologically rigorous and use other methods than those used so far, with careful monitoring of mite exposure and relevant clinical outcomes” [86].

Ideally, a study would ensure that it had high external validity by recruiting subjects whose asthma and domestic allergen levels are representative of the distribution of allergen levels and asthma severity in the community. To do this, a study would need to screen a large number of children, and only enrol a random selection of children who meet the inclusion criteria. This random selection will help to reduce selection bias and confounding. Once the children are enrolled, they should then be randomly assigned to one of the treatment groups.

The design of the study, and its interventions, should aim to minimise the burden on subjects and mask the subjects and researchers as much as possible. Yet, the interventions will have to be very intensive and radical, if they are to effectively lower allergen levels below the provocative threshold.

Ideally both active and control groups would be relocated to new homes which look identical in appearance. The active homes would be constructed with inbuilt and concealed allergen-reduction mechanisms, whereas the control homes would not have those features. Because these allergen-reduction features are in-built (and therefore hidden inside the walls/floors/ceilings) the trial can be double-blind. Another advantage of incorporating the interventions into the structure of the homes is that it should automate the operation of the interventions, which reduces the burden on subjects and ensures that the interventions do actually take place, which avoids the problem of poor subject-compliance. Allergens should rapidly accumulate in the control homes, but not in the active homes, and if the allergen-reduction hypothesis is correct, the asthma of children in both groups will initially improve due to the low baseline allergen loads in the new homes, and if the interventions do lower allergens in the active group but not the control group, then the control group's asthma should deteriorate, but the active group's asthma should remain stable and continue to improve. This would be an expensive trial, and subjects may not be willing to move to new homes.

The 'perfect' trial would use highly specific inclusion criteria to ensure that only people who are most likely to benefit from allergen-reduction are enrolled, such as people with high levels of specific-IgE [191], and requiring positive test to allergen-inhalation challenge as well as SPT<sup>V</sup>. In addition, it would require subjects to undergo steroid reduction, and lung-function tests to demonstrate reversibility of their obstruction, prior

to enrolment, to ensure they are capable of demonstrating any positive effect that allergen-reduction might have on asthma.

Future trials need to ensure that the placebo interventions do not reduce allergen levels to levels that are similar to those achieved by the active interventions. For example, Carswell et al found minimal difference in asthma outcomes between their two groups, but their placebo control group's allergen levels reduced substantially during the trial. An alternative way to overcome this is to have a factorial design that includes a group that receives no interventions. Ideally there would be two groups that receive no interventions: one group that gets regular home visits to collect dust samples and symptom diaries, and one group that does not receive any visits (to take account of the Hawthorne Effect) but there would need to be a way of measuring allergen levels and symptom data without the knowledge of the subjects.

Ideally, air sampling of allergens would be inbuilt to allow subjects to be masked to whether their allergen levels were being collected, and to enable the collection to occur without researcher's visiting the homes. One possibility would be to ask subjects not to clean their own homes, and to give all groups regular professional cleaning, and ask that the cleaners be allowed to visit the homes when the occupants were not home. Professional cleaners could be asked to clean the homes to a differential standard between the groups, and to collect dust samples. If control group subjects are asked not to clean their own homes this should prevent their allergens from decreasing due to control subjects over-cleaning which happened in previous trials.

It might be possible to have a positive control group as well as a negative control group, in order to clearly prove the allergen-reduction hypothesis, and to take into account the possibility that the allergen-levels might reduce in the placebo-control group even if the most theoretically inactive placebo is used. The researchers could lace the positive control group's homes with extra allergen to ensure their allergen levels remained at least as high as they were at baseline, or perhaps even to elevate them above baseline levels. This would be safe, because bronchial allergen challenge tests are safe in subjects who have a low level of allergy (as determined by intracutaneous test) [192], and these challenges have been used in many trials [193]. These trials used nebulizers and dosimeters to delivery very accurate amounts of allergen, whereas lacing bedding and/or carpet in the domestic environment with allergen is not as precise a way to present allergen to people and therefore, before this is done, it would be necessary to do it in an unoccupied model home, and to then vacuum the home to check how much higher the allergen levels were and whether the allergens had been evenly distributed or if the lacing was uneven.

Social support and encouragement from research staff, who develop a rapport with subjects and the use of more interesting ways of recording asthma symptoms should encourage better compliance with recording asthma symptoms and peak flows. For example, subjects could be given computers which are set up with software that has a highly rewarding appearance and which is appealing to children and parents. This software could be linked to the internet so that the results can be anonymized [194] and

analysed so that the results can be given to the parents in the trial so they can see how well their child is doing compared to the other children in the study. Qualitative data can be collected and analysed and the results reported back to parents on a regular basis throughout the trial so they can see what other people in the trial think of the study, and if they have any tips and suggestions that could help other subjects. This may lead to parents being more engaged and interested in the trial.

The period of analysis for each subject should be at least two years in order to take account of variation in seasons so that comparisons between groups can be restricted to ones that relate to the same season. The duration of two years will also allow for the long-term effects of the interventions to be tested, and will allow for sufficient allergen to build up in the placebo-control homes.

By following the suggestions laid out herein, which focus on automating and camouflaging the interventions, and increasing subject's buy-in, future studies may be able to give reliable guidance to families, clinicians, and policy-makers.

## **8 Conclusions and Policy Implications**

Despite optimistic results from small methodologically limited trials, which support the argument that allergen-reduction will improve asthma control in sensitised children, well conducted methodologically rigorous trials have not definitively proven this. Further research, which takes into account a wider range of potential causal factors, including the placebo effect, is required, before policy-makers can incorporate allergen-reduction interventions into evidence-based asthma treatment guidelines.

## 9 Tables and Figures

**9.1 Table 1: Range of possible causal and confounding factors, effect modifiers and outcome measures**

Potential causal factors of reductions in asthma severity (non-allergen)	Potential causal factors of reductions in asthma severity (allergens)	Potential effect-modifiers	Asthma outcome measures
Genetics, asthma severity at baseline, compliance with preventer medication, recent use of steroids, seasonal changes in air temperature and respiratory virus outbreaks, air pollution, strength of the sensitisation to allergens, number of allergens that the child is sensitised to	Moulds (aspergillus fumigatus, Hornodenrum cladosporium) Pollens (grass, tree), Rodents (mouse, rat), Pets (cat, dog, bird), Cockroach	Birth weight, Respiratory Distress Syndrome at birth, smoking, and exposure to environmental tobacco smoke, industrial air pollution.	Symptoms scores, quality of life scores, Peak flow, FEV <sub>1</sub> , FEV, FEV/FVC, eNO, days off school, plasma eosinophils,

## 9.2 Table 2: Systematic Review: characteristics of included trials

Authors, title	Study Design	Sample size	SPT/ ID	Sample Size enrolled / completed	Allergens	Severity	Active Intervention	Control Intervention	Duration of interventions	Outcome measures Conclusion	Intervention rooms	Duration outcome F/U
[110]	X C	14		12	HDM	NR	High altitude	Low altitude	8	No change in Histamine PC20-FEV1, significant change in eosinophilic markers. A similar study to this 1993 study was conducted in 1985 in 14 children and it found statistically significant improved PEF and FEV1 for the first 5 months which declined in the 6 <sup>th</sup> month. Progressive reduction in exercise induced bronchoconstriction throughout the 8 months. All subjects were able to discontinue their steroid and reduce their beta agonist.		
[90] **	B-B-P-C-R		SPT	62 enrolled 56 completed	HDM cat		Hot 60°C washing, Intervent cotton encasings coated on one side with polyurethane, acaricide: Acarosan powder on carpet, Acarosan foam on mattress, duvet, pillows, soft furnishings	Warm 40°C washing, cotton placebo covers, chalk dust instead of Acarosan powder, water spray instead of Acarosan foam	24	Less Der p 1 in Casella samplers (0 vs 29%, $P < 0.05$ ). Reduction in Der p 1 in Petri dishes (one dish on floor, one at pillow-height). Significant reduction in HDM allergen in bedding but not in bedroom carpets. In homes without cats greater reduction ( $P=0.03$ ) in mattress cat allergen. Improved asthma symptoms, reduced bronchodilator use, reduced bronchial irritability	Bed room only	24 weeks

[89] this is a report of the same study reported by [90]	B-B P-C R		SPT	49 / 70 enrolled	HDM cat	<p>Hot 60°C washing, Intervent cotton encasings coated on one side with polyurethane, acaricide: Acarosan powder on carpet, Acarosan foam on mattress, duvet, pillows, soft furnishings, remove soft toys. Surface HDM allergen levels were measured rather than µg/g dust.</p>	<p>Warm 40°C washing, cotton placebo covers, chalk dust instead of Acarosan powder, water spray instead of Acarosan foam, remove soft toys</p>	24	<p>100% (active) vs. 53% (placebo) reduction mite allergen on mattress at 6 weeks and 6 months, no effect of Acarosan on carpets, reduced BHR at 6 weeks but not at 6 months. Equally large increase in PEF for both active and control groups at 6 weeks. the mean daily PEF (as percentage of predicted for height) in the two groups at 6 weeks when house dust mite removal was most effective was 99.6% (SD 17.8) active, 98.9% (SD 14.5) placebo. Comparing the pattern of change in PEF variability or the frequency of PEF recordings below the 95% confidence interval (&lt; 87.6% predicted) for a normal child did not reveal a significant difference between the two groups at any time before or after the active intervention Improved FEV1 from 102.7% (SD 5.8) at baseline to 105.0% (10.2) at 24 weeks in the active group; in the placebo group there was a decrease from 101.8% (11.8) to 98.6% (15.3) over the same period. This difference between treatments was significant at 24 weeks (<math>P &lt; 0.05</math>). Slightly greater reduction in the histamine dose response slope 6 weeks after intervention in the active group (<math>f = -0.02</math>). but this was not evident at 6 months (<math>P = 0.23</math>). Percent of subjects who demonstrated BHR significantly reduced at 6 weeks (but not at 6 months) in the active group. At 24 weeks active group had improved FEV1, less bronchodilator use, lower symptom scores.</p>	Bedroom only	
[91]	S-B, P-C, non-P-C R		SPT	104 enrolled, 85 completed	HDM cockroach	<p>Active group: Allergen-impermeable bed and pillow covers, hot washing of bedding once a week, parents instructed on cleaning measures to control dust mites and cockroaches and were given cockroach bait. Home-visits at enrolment, 3, 8, and 12 months.  Placebo group: Allergen-permeable mattress and pillow covers, ineffective roach traps, and instructions to continue their normal practice of washing the bedding in cool or cold water. Home-visits at enrolment, 3, 8, and 12 months.  Non-placebo control Group: a home visit was only carried out 1 year after enrolment in the study</p>		52 / 52	<p>No significant difference between active and placebo either in asthma acute-visits or allergen concentration. When the children with mite allergy were considered separately, there was a significant correlation between decreased mite allergen and reduced acute asthma visits (<math>P &lt; .01</math>), and this was seen to the same extent in both the active and placebo groups. Only the non-placebo control group had an increase in acute visits (no allergen level data are provided for this group). The avoidance measures for cockroach allergen appeared to be ineffective, and the changes observed did not correlate with changes in visits</p>	Bedroom	One year follow up after baseline

[92] §	D-B, P-C R		Intra-dermal	26 / 23	HDM	Acarosan foam or wet powder. Applied to mattress, upholstery, and carpet.	Control foam consisted of tensids, perfume, propanebutane and water The placebo powder consisted of cellulose, water, and an antimicrobial preserving agent	52 / 52	Statistically significant improvement in clinical score (end of study compared to baseline) only for active group. Both groups significantly improved visual analogue scale for symptoms, and FEV1, no change in medication use. Der p I + Der f1 in patient mattresses between baseline and 12 months decreased 20% for the acaricide group and 17% for the placebo group, respectively (not significant). The decrease in guanine levels was significant only in the placebo-treated group. significant decreases in Der p I + Derf1 in the Acarosan-treated group (p < 0.01 for carpets; p < 0.05 for upholstery elements). The mean decrease in Der p I + Derf1 levels was 74% of the initial level for carpets and 67% for upholstery elements in the active group. In placebo-treated houses the Der p I + Der f I content also decreased in the two different samples, but was not significant Summary: Acarosan significant decreased mite allergens only in carpets and upholsteries but not in mattresses, possibly because of high allergen loads in mattresses at baseline.			Acarosan applied at baseline and 6 months
[93]	RCT		SPT	100	Cockroach, rodent	Home-based education, cockroach and rodent extermination, mattress and pillow encasings, and high-efficiency particulate air cleaner. Home evaluations at 6 and 12 months, clinic evaluation at 12 months, and multiple telephone interviews	Received the treatment after one year.	52	Levels of particulate matter 10 microm or smaller declined by up to 39% in the treatment group but increased in the control group (P < .001). Cockroach allergen levels decreased by 51% in the treatment group. Daytime symptoms increased in the control group and decreased in the treatment group (P = .04). Other measures of morbidity, such as spirometry findings, night-time symptoms, and emergency department use, were not significantly changed.			
[94]	RCT 3 groups			24 enrolled	SPT serum IgE	Acaricide group: Acarosan (benzyl benzoate) powder and foam for mattress and carpets. Encasings group: polyurethane lined cotton encasings for mattress, pillow, duvet, plus carpets were sprayed with 3% tannic acid on day 0 and at months 4 and 8	Matching placebo powder and foam, but there was no placebo alternative for the encasings.		Significant reduction in allergens with encasings but not with the active or placebo Acarosan or placebo. In the encasing-regimen group in which carpets were treated with tannic acid, and in the BB-treated group, there was a tendency to mite-allergen reduction. Only the encasing group had a statistically significant (but small) increase in histamine PD20. Only the encasing regimen achieved a significant reduction on mattresses up to 98%. PC2o significantly increased up to 4.5-fold in the encasing-regimen group after 8 months.			
[111]	Single-arm		SPT	36	HDM,	Air filtration of homes of atopic asthmatics using thermal convection caused by radiators for the adsorption of airborne particles	No control group	52	The filters extracted and captured allergens from HDM and mould. 55% of subjects reported an improvement in their asthmatic symptoms. The paper is in German so I could not determine if this self-report had been independently validated.			

[95]	RCT, RAST		RAST	23 Aged 5 to 18	dust mite, cockroach, and cat allergens	Encasing mattress, box springs, and pillows in allergen impermeable covers (cotton with urethane membrane); weekly hot water wash of bed linens (mattress pad, sheets, pillow cases, blanket/duvet); replacement of bedroom carpet with bare-surfaced flooring; and 3% tannic acid spray to living room carpet every 3 months.	Polycotton (without urethane membrane) permeable encasing for mattress, box springs, pillows; cold water wash (mattress pad, sheets, pillow cases, blanket/duvet), and water spray for carpet		Allergen levels fell > 3-fold in many active and placebo homes. Although 8/11 active homes had elevated floor dust mite allergen levels initially, only 6/8 had reduced levels below 2 µg/g after the carpet was replaced with vinyl linoleum or hardwood flooring. In five of six homes where the initial bedding dust mite allergen levels was elevated, the levels fell after intervention. Spirometry showed continued significant obstruction despite overall improved PEFR recorded on diaries. Children in the active group had improved PEFR at 3 and 6 months after intervention (P < .04, P < .05, respectively). Six of seven children in the study who were sensitized and exposed to dust mite allergen demonstrated improved PEFR at 3 months when allergen levels fell in both bedding and bedroom floor. There were significant improvements in PEFR in active group at 3 months and 6 months post-intervention (+13.4% vs -1.2) and (+15.1 vs -4.4), respectively. There was no difference in FEV1 or methacholine challenge. Six children (four active and two placebo) were readmitted to hospital during the study.	Dust collected from bedding, bedroom floor, living room floor, upholstered furniture, kitchen floor.
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[96]	Open-label p-C RCT	Not an inclusion criterion, but serum IgE levels were measured after enrolment	62 enrolled / 51 completed	Mould	Action plan, education (on asthma action plan, medication use), and individualized problem solving, home cleaning information including how to overcome any barriers to compliance with cleaning procedures. Household repairs were done, including reduction of water infiltration, removal of water-damaged building materials, and heating-ventilation-air-conditioning alterations. A 24-hr telephone hotline, answered by trained nurses or physicians, was made available to all patients for questions regarding acute asthma management Telephone follow-up calls occurred at 2, 4, 8, and 10 months and follow-up visits at 6 and 12 months after randomization	Action plan, education (on asthma action plan, medication use), and individualized problem solving, home cleaning information including how to overcome any barriers to compliance with cleaning procedures.. A 24-hr telephone hotline, answered by trained nurses or physicians, was made available to all patients for questions regarding acute asthma management. Telephone follow-up calls occurred at 2, 4, 8, and 10 months and follow-up visits at 6 and 12 months after randomization	Permanent repairs. Regular education.	Only a few of the allergen levels significantly reduced. Total visible mold scores, were significantly lower in the remediation group compared with the control at both EV2 and EV3. The reduction in mold scores from baseline to EV2 and EV3 were greater in the remediation group compared with control (P=0.07). The reduction in endotoxin between baseline and the EV2 sample (but not the EV3) was significantly greater in the remediated compared with the control group. There was a trend toward a greater reduction in mouse allergen levels in the remediated group compared with control at EV3, surprisingly there was a trend toward greater $\beta$ -glucan reduction in the control group compared with remediation at EV3. Significant within-group reduction was seen at EV3 for mouse urine in the remediated group. Der p 1 levels were significantly increased at EV2 in the control group. The remediation group had a significant decrease in symptom days after remodelling, whereas these parameters in the control group did not significantly change. After remediation the remediation group had a lower rate of exacerbations compared with control asthmatics (1 of 29 vs. 11 of 33, respectively, $p = 0.003$ )	All points of moisture were remediated. Mould measured in basement, kitchen, TV/living room, bathroom, child's bedroom, attic, and other bedroom	End of study was
[97]	RCT	SPT IgE levels	30 Aged 5 to 18	Subjects were	Sealing cracks and crevices in the structural envelope of each apartment, applying low-toxicity pesticides, thoroughly cleaning surfaces, eliminating food and water sources, and educating household members about maintaining a pest-free environment.	Identical treatment as the active group, but delayed by 8 months after baseline.	Not reported in this article – results were still pending.			

[98, 99]	RCT O.L. BAPE			274 randomised		High-intensity group. Allergen control pillow and mattress encasements, low-emission vacuums, commercial-quality door mats, cleaning kits, referral to smoking cessation counselling, cockroach bait, rodent traps and assisted cockroach and rodent eradication. Community health workers provided individualized action plans, and 4 - 8 home visits for one year for education, social support, encouragement of allergen-reduction procedures (Social Cognitive Theory, Transtheoretical model), and advocacy for improved housing conditions.	Low-intensity group received the initial assessment, home action plan, limited education during the assessment visit, and bedding encasements. After outcome data were collected one year later, control participants received all the high-intensity interventions	52	Significant improvement in paediatric asthma caregiver quality-of-life score and asthma-related urgent health services use. Non-significant fewer asthma symptom days (P= .138). Participant actions to reduce triggers generally increased in the high-intensity group. The projected 4-year net savings per participant among the high-intensity group relative to the low-intensity group were \$189-\$721. However, only rodent counts reduced significantly, there was no significant reduction in cockroaches or mould.		
[100]	R S-B P-C	SPT		35 (aged 13 to 60)		Active allergen avoidance treatment (n = 17) allergen-impermeable covers over the mattress, pillows and duvet and spraying the remaining bedding, as well as the carpets and furniture, with a tannic acid/acaricidal spray	Placebo (n = 18) Inactive spray, matched in appearance to the active spray, to be applied in the same manner as for the active group. No placebo equivalent for the impermeable covers that the active group received.	26	Daily record of symptoms and PEFr, 3 monthly spirometry and broncho-provocation challenge. Dust samples from bed, the bedroom floor living room floor at 3 monthly intervals and 2 weeks after the treatment. Mean bed HDM allergen levels at two weeks after the allergen avoidance treatment were reduced to 29% of baseline in the active group and 61%, in the placebo group (P=0.038 btw groups), but was not significantly different at 3 or 6 months. There was also no significant effect of the allergen avoidance treatment on symptom scores, peak flow variability, lung function or PD20 P > 0.1).	Bedroom, living room.	Allergen-reduction lasted 6 months, but no improvement in asthma.
[101]	Three-way	SPT		49 enrolled, 22% LTFU	cockroach	Three parallel groups: A. professional cleaning with insecticide bait traps B. professional cleaning with placebo (non-insecticidal) bait traps C. no cleaning or bait traps All groups were educated on keeping food covered, removing food scraps, and prompt washing of dishes.		Trial lasted 11 weeks, bait was laid twice, professional	No clinical asthma outcomes were measured, the only outcome was reduction in cockroach levels. Median cockroach counts were reduced in the homes treated with insecticide bait traps but not in other groups. None of the three groups had a significant reduction in allergen levels. Sub-group analysis found a significant reductions in allergen levels in the kitchen in homes that received professional cleaning and had higher initial cockroach counts (54), regardless of whether bait traps had insecticide. At end-of-study over half of all kitchens and bedrooms in cleaned homes had cockroach allergen levels above 8U/g.	Intervention to whole house, samples from bedroom, bed, kitchen.	Bait given twice during the 11 week study period
[66]	RCT	SPT		937	HDM Rodent	Physician feedback bimonthly. 5 – 7 home visits for education and reinforce intervention behaviour. Allergen-impermeable mattress, pillow, bed-base covers; HEPA vacuum cleaner; HEPA air filter for cat, dog, ETS, mould; professional pest control for cockroach	6-monthly visits for collection of outcome measures only. No placebo.	52 for intervention + 52 follow-up	Compared to control group, active group had statistically significantly fewer days with symptoms (3.39 vs. 4.20 days per fortnight, in intervention year, (2.62 vs. 3.21 days per fortnight during follow-up year; greater declines in Der f1 in the bed, and bedroom floor, Der p in the bed, and Bla g cockroach allergen on the bedroom floor (P<0.001). Reductions in the levels of Bla g and Der f1 on the bedroom floor were significantly correlated with reduced complications of asthma		

[108] †	Non-randomised controlled trial		SPT, Serum IgE	36 children in 24 homes, aged ≤7yrs mean 3.8 yrs	HDM		Regular counsel at clinic visit regarding cleaning and second-hand tobacco smoke avoidance plus monthly intensive home visit education and advice (>60 minutes per visit) for avoiding HDM (all home occupants to wash at room temperature bedding encasings (typical Japanese bedding) more than once a week, vacuum bedding, bedroom floor, living room floor more than once a week. Remove from house: soft toys, furred pets, carpets. This group was divided into two sub-groups: Group A were atopic, Group B were non-atopic.	Standard guidance (10 minutes per patient) at standard-care clinic appointments, regarding cleaning and second-hand tobacco smoke avoidance. This group was divided into two sub-groups: Group C were atopic, Group D were non-atopic.	52	Seventeen children with asthma were found to be sensitized to HDM allergens, and the remaining 19 children with asthma were found to be sensitized to neither HDM nor 7 common allergens. Eleven HDM-positive (group A) and 13 HDM-negative (group B) children with asthma received home visit education and advice for allergen avoidance. 6 HDM-positive (group C) and 6 HDM-negative (group D) children were enrolled in the control group. The reductions in Der p 1 and Der f 1 were 20.1, 10.3, 0.9, 2.2 in Groups A, B, C, D, respectively (statistically significant for Groups A and B). Focussing on the atopic children: Der p 1 and Der f 1 reduced by 20.1 in the active group (A) compared to 0.9 µg/g in the control group (C). Intensive education and advice in atopic children resulted in significantly fewer asthma attacks 2.5 compared to 4.7 in active (A) vs control (C) groups. There was a similar effect on asthma attacks in the non-atopic active versus control groups. Both groups had a significant reduction in their theophylline dose.		One year
[102]	R, D-B, P-C		SPT, RAST	51 enrolled, 46 completed,	HDM	GINA	Natamycin sprayed onto mattresses (to kill the fungi that support house dust mite wellbeing)	Placebo spray	Spray every 2/52 for 3/12.	No effect on Der p 1 in mattress, histamine bronchoprovocation PD20, or lung function spirometry.	Bedroom	No improvement in one year.
[56]	R, D-B, P-C		SPT	38 enrolled, 30 completed, aged 11 to 42 ± (mean age 29)	HDM	Moderate to severe	Allergen impermeable covers for mattresses, pillow, bed covers. Wash sheets weekly at 60°C. Bare floored bedrooms.	Placebo covers made by the same company Cara C'air (Allergy Control AC btm Velsbroek, Netherlands), which made the active covers to match the appearance of their active product. Bare floored bedrooms.	52	Significant reduction in mattress Der p 1 mattresses in the active group after 1 year compared with baseline, with no reduction seen in the placebo group. In both groups there was no significant improvement in PC20 histamine. Quality of life improved similarly in both groups. There was a significant decrease in nasal symptom score within the active group (end of study compared to baseline), but there was no significant difference between the active and control groups. No changes in morning and evening peak flow values, peak flow variability, nor in the use of rescue medication were found in either group. Allergen impermeable encasings caused significant reductions in Der p 1 concentrations in carpet-free bedrooms, however this did not translate into improved asthma. .	Bedroom	1 year. Significant reduction in Der p 1 began after 4 months and continued till the end of study 1 year later.

[105]	Randomised double-blind placebo-controlled	SPT	32 (22 male) mean (SD) age 12.8 (1.8) years..			A total of three groups: An active double-blind group (A) n=14, a placebo group (B) n=10 which was double-blind and received all the same interventions as Group A except they had placebo benzyl benzoate, and a non-blind control group (D) n=8 which got no active or placebo benzyl benzoate. All children visited the Italian Alps in a house dust mite free environment, for at least 2 months prior to the start of the study. During their 9 month stay in the Alps, they returned for two short periods of 20 and 10 days each, to their homes at sea level. All patients received education on removing all carpets, dust-collecting textile objects (furniture, curtains, toys), synthetic material in the bedroom, combined with daily vacuum cleaning and wet-mopping. No feather pillows were allowed and no patient was using any kind of mattress covers before and during the study period. In Group A, Acarosan was sprayed on mattresses in child's bed at home, in group B, placebo Acarosan was sprayed on mattresses in child's bed at home, in Group C nothing was sprayed.	Two applications of benzyl benzoate, but only to beds at their homes where they only	Benzyl-benzoate foam sprayed onto mattresses was no more effective than placebo in reducing the level of house dust mite recovered from patients' mattresses, or in reducing bronchial hyperreactivity and IgE concentration in serum and nasal secretions	Bedroom.	No improvement
[104]	Double-blind randomized placebo controlled trial	ID	45, aged 18 to 45 years old			Group 1: active air-cleaners in living-rooms and bedrooms. Group 2: placebo air-cleaners plus allergen-impermeable mattress covers. Group 3: active air-cleaners plus allergen-impermeable mattress covers.	26	Substantial amounts of Fel d 1 and Der p 1 were entrapped by air filters. Only beds that had mattress covers (Groups 2 and 3) had decreased dust and HDM allergen levels. Only Group 3 had a statistically significant increase in PD20 histamine dose (increased from 5.96 to 9.02 mg·mL <sup>-1</sup> ). There was a trend to lower peak flow variability in Groups 2 and 3, yet the absolute change was not statistically significant, interestingly the reductions were correlated with the amount of dust and house dust mite allergen collected in the filters. Only Group 2 had decreased eosinophil counts and IgE (at 3 and 6 month). Air filters gave no benefit as monotherapy (Group 1), and among those who got encasings, those who got the placebo filter (Group 2) had improved eosinophils and IgE but those who got the active filters did not. So filters+encasings are good for PD20 but not good for eosinophils and IgE (this is of dubious biological plausibility). Subjects who got both active air filters and active encasings, or got active encasings with placebo filters, did better than those who got only air filters.	Living-room, Bedroom.	
[107]	RCT (*)	SPT	40 completed symptom diaries. 27 children (4-16 yo mean age 9.7) 13 adults	HDM	Mod - severe rqr	(1) mechanical ventilation and a HEPA filter vacuum cleaner ; (2) mechanical ventilation alone; (3) an HEPA filter vacuum cleaner alone; and (4) no intervention. Vacuum bedroom living room daily, rest of house weekly	Monitored for 12 months	Mechanical ventilation (MVHR) significantly lowered humidity levels, which was significantly associated with reductions in both mite numbers and Der p 1 in bedroom carpets and some other areas. These reductions were enhanced by the addition of a vacuum cleaner. There was a significant trend with MVHR (but not HEPA) for an improvement in histamine PD20. During winter only groups 1 and 2 had humidity values of less than 7 g/kg for sustained periods of time. No change in eosinophil activation. There was no significant change in spirometry or symptom reported in any group.	Whole house	12 months

[109]	O-L, P-C		SPT 4mm	16 age 5 – 15		Thorough cleaning (non-HEPA vacuum, wipe surfaces) of living rooms in the homes of 16 atopic asthmatic children were thoroughly cleaned and treated with Allersearch DMS (alcohol-based benzyl tannic acid complex)	Thorough cleaning only.		In 13 of the 16 active homes there was a statistically significant reduction in Der p I in carpet (but not below 2 µg), and in 11 homes Fel d I was significantly reduced. Changes in allergen concentrations in soft-furnishing dusts were not significant. No reductions in allergens occurred in the control group. Highly statistically significant reductions in SPT reactivity to subject's own dust. No asthma symptoms were recorded.	Whole home.	2 weeks
[106]	D-B, P-C, R, X		SPT 3mm wheal	20 median age 9 (range 3-11)	HDM	Active ioniser for 6 weeks.	Placebo ioniser for six weeks.	6 week per intervention	Statistically significant reduction in airborne Der p I concentrations during the active period compared with the placebo period, but this is shown as a graphic rather than as a mean value. No significant change in PEF, symptom scores, or use of medication. There was an increase in night time cough which almost reached significance during the active period.	Bedroom, living room	
[103]	D-B, P-C, R		SPT	70 invited, 62 received treatment, 56 completed	HDM	Bedroom carpet, mattress, duvet and pillows treated with Acarosan, then encased in vapour permeable waterproof fabric (Intervent--cotton coated with polyurethane) Soft furnishings treated with Acarosan,. Soft toys washed at 60°C	Bedroom carpet, mattress, duvet and pillows treated with placebo-Acarosan, and encased with cotton covers. Soft furnishings treated with placebo-Acarosan,. Soft toys washed at 40°C	24	Median reduction of 480 ng (100%) in mite allergen from the mattress vs 215 ng (53%) reduction in placebo-treated group by 6 weeks and remained reduced for the 24 week duration of the study. The Der p I content of the active group's bedding was always less than the placebo group after treatment (P < 0.01). The acaricide, which was applied to carpets and mattresses underneath the encasings, was ineffective in reducing allergen content in both areas.	Bedroom	6
[112]	RCT		RAST ***	410 enrolled 161 randomized, 34 completed 12-month assessment	HDM, cockroach	NR	Control group received the same interventions as the active group, but were delayed by 12 months. Community health workers provided information, education, and assisted with asthma management and environmental interventions for cockroach (hydramethylnon gel, education about food handling) and HDM (allergen-impermeable encasing mattress, bed-base, pillows; instruct on washing and cleaning). Advised to smoke outdoors. Professional cleaning to remove allergens at baseline, If necessary intervention to reduce moisture, mould, and pets. Environmental health worker gave verbal and written information at baseline, 2, 6, and 10-month home visits.	52	Dust collected from floor, bedding, upholstered furniture in bedroom and room where child spends most time during the day. End-of-study levels of HDM allergen increased 163% over baseline in control group, but remained stable in the intervention group (p<0.05). Cockroach allergen was lower in the active group (p<0.05) but the difference was not large by the end of the study. All outcomes are presented as percentages of baseline levels, or as graphs, no numerical mean values are provided for any outcome. No difference in total asthma scores between the two groups at any time-point. Post hoc analysis showed a statistically significant difference in one of the sub-sections of the Asthma Score, called the functional component, for which the intervention group's score showed better function than the control group..	Bedroom, kitchen, whole house	No durable outcome.

**Notes:**

† Some of the statistical calculations were not in the Nishioka paper, and so I calculated them (see Word File “Nishioka working out the statistics”

‡ The only children in this study (two aged 11, one 12, and one 17 years old) were all in the control group despite randomisation.

§ Note age range of subjects 13 to 58 years (mean 35 ± 14.9 years).

R = randomised; S-B = single-blind, D-B = double-blind; BAP = Blind Allocation Procedure; C = controlled, X = cross-over, P-C = placebo-controlled, NP-C = non-placebo-controlled, SPT= skin prick test; ID=intra-dermal skin prick test, btw = between, NR = not reported.

(\*) this was not an ideal randomisation – if a house could not be fitted with a MVHR then it was randomised to one of the two non-MVHR groups.

(\*\*)this is a report of the same study reported by [112]

(\*\*\*)RAST results were not part of inclusion criteria

**Table 4 Overview of results and set up of controlled mattress cover studies**

Author	Study set up						Parameters					
	No of patients	Age	Design	Duration (weeks)	Carpet	Acaricide	Der p 1	PC <sub>20</sub> histamine	Symptoms	Peak flow	Medication	
Sarsfield <sup>28</sup>	14	Children	open c	52	-	-	+	ND	+	ND	ND	
Murray <sup>26</sup>	20	Children	pl c	4	-	-	-	+	+	ND	ND	
Walshaw <sup>29</sup>	50	Adults	pl c	52	+	-	+	+	+	ND	ND	
Gillies <sup>23</sup>	24	Children	open c	12	+	-	-	-	-	ND	ND	
Ehnert <sup>8</sup>	24	Children	open c	52	+	+	+	+	ND	ND	ND	
Marks <sup>22</sup>	35	Adults	r c	26	+	+	-	-	-	-	ND	
Weeks <sup>20</sup>	56	Children	r db pl	24	+	+	+	ND	ND	ND	ND	
Carswell <sup>19</sup>	70	Children	r db pl	24	+	+	+	-	ND	-	ND	
van der Heide <sup>9</sup>	59	Adults	open c	52	+	+	+	+	ND	ND	ND	
Frederick <sup>10</sup>	31	Children	r sb pl	12	+	-	+	-	-	-	-	
van der Heide <sup>9</sup>	45	Adults	open c	26	+	-	+	-	ND	ND	ND	
Cloosterman <sup>14</sup>	29	Adults	r sb pl	6	+	+	ND	ND	+	-	ND	
Sporik <sup>21</sup>	85	Children	open c	78	+	+	-	ND	ND	ND	ND	
Cloosterman <sup>11</sup>	157	Adults	r db pl	20	+	+	+	-	-	-	ND	

open c=open controlled; pl c=placebo controlled; r c=randomised controlled; r db pl=randomised double blind placebo controlled; r sb pl=randomized single blind placebo controlled; ND=not done; +=significant change; -=not significant change.

**9.3 Table 3: Systematic Review: summary of outcomes of systematic review**

	PEFR variability	PEFRmorning	PEFR evening	Mean daily PEFR	PEFR below 95%	PEFR at clinic visit	FEV1	FEF 25-75	FEV1/FVC	PD <sub>20</sub> or PC <sub>20</sub>	Histamine DRS	Histamine BHR	ECP	EPX	total IgE	Eosinophilia	MPO
[110]										±			+	+	+		±
[89, 90, 103] *	±	±		±	±		+				±	±					
[91]	<b>nr</b>	<b>nr</b>		<b>nr</b>	<b>nr</b>												
[92]		±	±	±			±	±									
[93]							±										
[94]										++							
[111]																	
[95]				++			±			±							
[96]						± <b>p</b>			± <b>p</b>								

[98]																		
[100]	±	±	±				±					±						
[66]	±	±	±	±			±	±										
[108]																		
[102]				±	±		±			±								
[56]	±	±	±							±		±						
[105]										±							±	
[104]	± pp	±	±							+							+ **	+ **
[107]	±	±	±				±	±	±	±	±					±		
[109]																		
[106]	±	±	±															
[112]																		

	Asthma Symptom score†	Nnasal symptom score	Asthma exacerbation rate	Asthma attacks	ADL score	Daytime wheeze	Cough	Reduction SPT	Use of bronchodilators	Use of corticosteroids	Acute healthcare	Quality of life score	Disturbed sleep	Disruption of plan	Days off school	Physical activity score	Reduction in allergen ‡
[110]																	+
[89, 90, 103] *	+ §					±	±		+	±							+++
[91]											++						++
[92]	±					+			±	±							++
[93]	+										±	±				±	++
[94]																	+++ +
[111]	(+)‡ ‡																(+)‡ ‡
[95]											-						++

[96]	++		++							(+)	¥						(+)#
[98]	+				+++			±††	±	+++	+++				±		nd
	††										∅						
[100]	±									±							±
[66]	+++				+++	+++				+++		+++	+				+++
[108]	(+)			+						(+)							+
[102]																	±
[56]	±	+							±			±					+++
[105]																	±
[104]																	+
[107]	±								nr	nr							(+)¶
[109]								++									(+)¥
																	¥
[106]	±						-		±	±							+ ∅∅
[112]	±				(+)				±	±	±						++
					«»												

+ small improvement, ++ medium improvement +++ substantial improvement

- small deterioration, -- medium deterioration, --- substantial deterioration

± no change (change is defined as a change compared to the control group)

Where the above symbols are surrounded by brackets ( ) they are of doubtful plausibility (due to 1. study design flaws such as non-ideal outcome measure, or 2. low biological plausibility)

ADL – Activities of Daily Life

nr not reported, nd not done (not part of the study design)

\* the results of this study would actually be non-significant if they had been adjusted for multiple comparisons

§ these outcome measures were significant at 2 and 24 weeks but not at 6 weeks, which suggests that it was not caused by allergen reductions because they did not rise at 6 weeks and go back down again at 24 weeks.

† the symptoms score of the scale used in the paper (such as the total scores of the individual symptom scores, such as wheeze, cough etc)

‡ reduction is baseline compared to end to study within group (not between groups)

# reduced ad hoc mould score, but not statistically significant reduction in endotoxin or  $\beta$ -glucan.

In my assessment of whether or not there is evidence of improvement, I have restricted my judgement to the subjects who were sensitized and did experience allergen-reduction and I have also restricted myself to biologically plausible and longer-term outcomes.

I excluded non-controlled studies since it is not possible to tell whether the allergen-reduction caused the improved symptom, hence Fischer et al is not in this table.

¥ statistically significant in an as-treated analysis, but not statistically significant in an as-randomised intention-to-treat model.

Ð very small sample size, there was a non-significant improvement in pulmonary function outcomes which may have been significant in a larger sample size.

Ø caregiver quality of life (all others in this column are the child's quality of life)

†† not significant ( $p=0.138$ ), but a small sample size

ÐÐ no significant reduction in peak flow variability between the groups as a whole, but there was a significant relationship between the amount of Derp1 captured by the filters and reduction in peak flow variability among subjects who also received allergen-impermeable encasings

\*\*the reduction in eosinophilia and IgE was only in the group that had placebo air cleaners and impermeable encasings, those with active air filters with/without encasings had no improvement in eosinophilia.

¶ only a statistically significant trend to reduced Derp1 in bedroom carpet, mattress and sofa when measured as Derp1 per square metre, but not significant in mattress or living room carpet when measured as Derp1 per gram of dust.

¶¶ no significant difference, but there was a non-significant trend to improvement

¥¥ this reduction was an artefact

∅∅ statistically significant reduction, but absolute levels of Derp1 not reported so cannot evaluate whether levels were reduced below the provocative threshold

«» this statistically significant change in Functional Severity Score was not an a priori analysis – it was done on the basis of advice from non-blind researchers, and since it is a subjective outcome measure, and the trial was open-label, it is of dubious validity.

I have done a nice table to put in here, which I have saved in a separate Word File called “Table of systematic review results”

#### 9.4 Table 5: Skin Prick Test results for enrolled subjects (plus one sibling)

The prevalence of skin prick test reactions for the ten subjects (plus the brother of one of the subjects) is provided in the table below:

<i>Subject Name</i>	<i>Derp1</i>	<i>Derf1</i>	<i>A fumigatus</i>	<i>Dog Hair</i>	<i>Cockroach</i>	<i>Cat pelt</i>	<i>Mouse</i>	<i>Rat</i>	<i>Penicillium9</i>	<i>Alternaria tenuis</i>	<i>Hrnodendrum cladosporium</i>	<i>Garss Mix</i>
<i>KK</i>	+	+										+
<i>JP</i>	+	+		+								+
<i>HP</i>	+	+				+						+
<i>KT</i>	+	+										
<i>AV</i>	+	+	+		+					+	+	+
<i>RW</i>	+	+				+					+	+
<i>JWM</i>	+	+			+	+		+				+
<i>JWP</i>	+	+			+	+	+			+		+
<i>AY</i>	+	+										

<i>AR</i>	+	+				+						+
<i>AB</i>	+	+				+						+
<i>Total</i>	<i>11</i>	<i>11</i>	<i>1</i>	<i>1</i>	<i>3</i>	<i>6</i>	<i>1</i>	<i>1</i>	<i>0</i>	<i>2</i>	<i>2</i>	<i>9</i>

(note that JWM and JWP are siblings)

### 9.5 Table 6: Allergen exposures

<i>Name</i>	<i>Mould</i>	<i>Cat</i>	<i>Dog</i>	<i>Cockroach</i>	<i>Rat</i>	<i>Mouse</i>	<i>Tobacco</i>
<i>KK</i>	+						
<i>JP</i>							
<i>HP</i>	+					+	
<i>KT</i>		+					
<i>AV</i>	+			+			+
<i>RW</i>	+		+				

<i>JWM</i>							+
<i>JWP</i>							+
<i>AY</i>	+	+	+	+		+	
<i>AR</i>	+	+		+	+	+	
<i>AB</i>	+	+					
<i>Total</i>	<i>7</i>	<i>4</i>	<i>2</i>	<i>3</i>	<i>1</i>	<i>3</i>	<i>3</i>
<i>number</i>							

Note that in the two families in which the parent(s) smoke, the smokers always smoke outside the house.

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## **11 Appendices**

### ***11.1 Appendix 1: Corollary with Air Ionisers***

It is useful to briefly look at air ionisation to improve asthma, rather than allergen-reduction, since it is easier to be dispassionate and rational about an intervention that is not the focus of one's interest, than it is to be logical and clear-thinking about a topic, such as allergen-reduction, into which one has invested hope and emotion. In the 1950s a number of studies were done to see if air ionisation would improve atopic disease such as asthma and hayfever. A preliminary study of the effect of ionised air on pollinosis employed no control group and found that 17 of the 27 subjects had improved symptoms, and this was taken as evidence that negative ionization of the offending air-borne substances, such as dust, pollen, fungi, viruses and bacteria diminishes their allergic toxicity by changing their electric potential, thus rendering them temporarily inactive [112]. This biologically implausible rationale was later tested using a control group, however it only had 15 subjects in the control group, 108 subjects in the active group, did not randomise subjects, did not blind the researchers who had to rate hayfever symptoms on a subjective scale (none, minimal, moderate, marked), and only exposed subjects to 12 – 50 minutes of ionised air, which casts doubt over the validity of its claim that ionised air temporarily improved symptoms (no statistical analysis was performed) [195]. The absolute necessity of having a control group in order to be able to make any sense of a

study's findings, is clearly illustrated in studies of air ionization to improve asthma [196]

I. No control group was used in this study of air ionisation in only 7 asthmatics, and the researchers attempted to take account of the placebo effect by having a series of sequential interventions, with all 7 subjects being exposed to the placebo period first. All subjects were exposed to the intervention in the same sequence, with no randomisation and no reverse of the order of the cross-over.

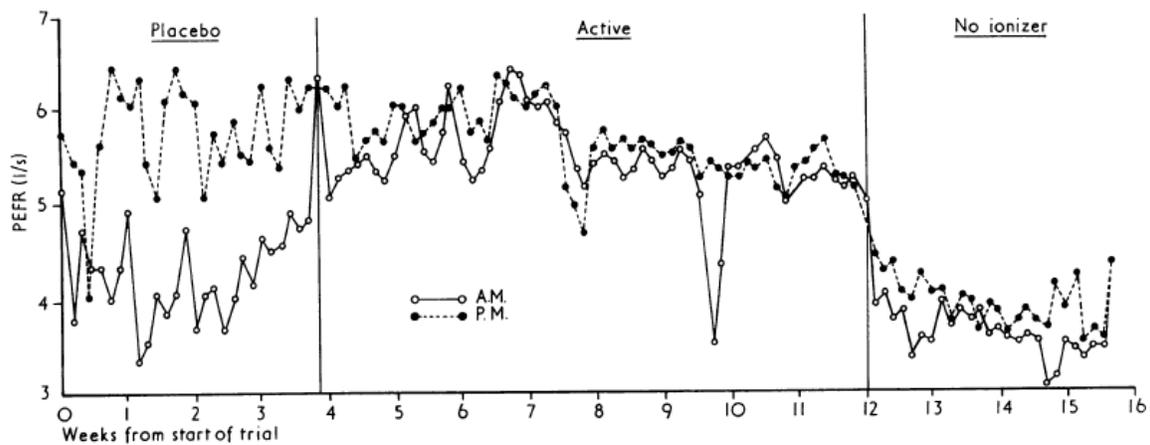


FIGURE. Patient 6. Morning and evening PEFr v time

In 1966 an Israeli team compared air ionisation in 19 hospitalised children (13 with and 6 without asthma) to no air ionisation in 19 hospitalised children (19 with asthma) [197]. The authors picked out one subjects whose airway responsiveness matched their hypothesis, and did not give data on the other 18 subjects, and no statistical tests were done. Another Israeli group re-examined the effect of air ionisation using a somewhat more scientifically rigorous method (double-blind placebo controlled, cross-over, but *not* randomised design) in 17 children with exercise-induced asthma [198]. The 17 children were exposed to ionised air on one day, and ordinary air on the other day after which their airway hyperactivity was measured by exercise in 11 children and histamine in 10

children. The authors report that exercise-induced asthma was attenuated with ionised air ( $p < 0.015$ ), however this is at odds with the table of their results which states that the mean fall in FEV1 was 29 (S.E. 5) and 21 (S.E. 3) in the ionised and control air, respectively, which means that the drop in FEV1 could have been as low as 24% in the ionised air group and as high as 24% in the control group (no significant difference). The studies outline above all have fundamental flaws in their study design, most notably of which was their failure to use a control group, and hence their conclusions that air ionisation improves asthma are not secure.

As early as 1960s there was a high-quality (randomised double-blind cross-over) in 17 subjects which found no effect of ionisation, but its conclusions were limited by its low power [199]. An Australian study in 1983 had a robust study design (double-blind, randomised placebo-controlled cross-over trial with sufficient wash-out period) and used a generous duration of treatment, and was therefore able to conclusively show that air ionization had no effect on any of a number of asthma outcome measures [200].

## ***11.2 Appendix 2: Study design and causality***

This appendix outlines study designs from Campbell and Stanley and contains a description of the variety of possible causal factors for diseases, and the ways in which appropriate study designs can ensure that it is possible to reliably determine which of the factors is a causal one.

## 11.2.1 Experimental and Quasi-Experimental Designs for Research

Campbell and Stanley outline a number of threats to the ability to detect a genuine causal association in the context of education, which are similar to those in community-based allergen-reduction research:

1. **Maturation.** The nature of children's asthma, and their ability to comply with trial requirements (e.g. answer questionnaires, conduct peak flows, avoid dust) alters with the passage of time. Improvements in asthma as a result of this maturation process can be mistakenly interpreted as evidence that the trial interventions improved children's asthma. This is avoided by using a control group, in which the same maturation process will occur.
2. **Reactivity.** Improvements in asthma may not be due to the allergen-reduction interventions, but instead be the result of a response to some other aspect of the trial, such as the filling in of questionnaires, increased awareness of asthma through recording of peak flows and symptoms diaries, observation by research staff (Hawthorne Effect), the novelty of having important respected strangers (researchers) visit the home and pay close attention to the families' circumstances (Pygmalion Effect).

A threat to external validity comes from the sensitizing effect of pre-tests. If pre-testing is an important part of the trial, the pre-test might be a necessary pre-cursor in order for the improvement in asthma to occur, and if only the interventions, but not the pre-test are conducted in the later nation-wide protocol, then the same improvements in asthma may

not eventuate. For example, if an allergen-reduction trial had a complex questionnaire and series of lung-function tests at baseline, and found that their interventions improved asthma, it is possible that the allergen-reduction interventions only improve asthma in children who have completed complex questionnaires and lung-function tests first.

Campbell and Stanley utilise the following notation:

R - randomised

O – observation

X – intervention

This notation can be used to describe the procedure of a trial, as a series of the above symbols written from left to right in the order in they occur in time. For instance, R O<sub>1</sub> X O<sub>2</sub> is shorthand for: “initially the subjects were randomised, then data were collected (observation 1), then the intervention was performed, and subsequently there was another data collection (observation 2).”

The following design has reduced generalisability due to the pre-test observation which may be responsible for any beneficial outcome:

Group 1: R O<sub>1</sub> X O<sub>2</sub>

Group 2: R O<sub>3</sub> O<sub>4</sub>

To overcome the clouding of the determination of causality by the potential effect of the pre-test, one must undertake a large four-way factorial design called The Solomon Four-Group Design [75]:

Group 1: R O<sub>1</sub> X O<sub>2</sub>

Group 2: R O<sub>3</sub> O<sub>4</sub>

Group 3: R X O<sub>5</sub>

Group 4: R O<sub>6</sub>

The Solomon Four-Group Design, is logically sound, and provides robust proof of causality, but it requires substantial resources (money, staff, time) and has feasibility problems (such as how to enrol subjects and then collect data from them without observing them or having contact with them until the end of the study).

### **11.2.2 Control group and random allocation**

A control group is required to take account of plausible competing hypotheses for what might be the cause of the outcome under investigation. One must ensure that the control group and active group are identical with respect to all factors that could be plausible alternatives to the hypothetical causal factor under investigation. The plausibility of the argument that X is the cause of Y, increases as the number of potential alternative factors competing with X decreases. This is termed the rule of parsimony [75]. The rule of parsimony requires the study design to restrict the number of ways in which the control and active groups differ to as few factors as possible. Ideally the only difference in exposure between the two groups would be the exposure to the intervention under investigation, but this is rarely practical in the real world.

The use of a control group should ensure that each of the rival hypotheses can be tested and found to be null. Some rival hypotheses relate to all trials of causality in general, others relate specifically to allergen-reduction. Two rival hypothesis exist for all trials: 1. regression to the mean, and 2. natural history. Regression to the mean is a statistical phenomenon that when an extreme outcome is observed, it will tend to become less extreme on repeat observation. Thus in an uncontrolled study, it could happen by chance that an extremely high asthma symptoms score was observed at baseline, so that when this symptom score is re-administered at baseline, it is likely that the score will be lower. This function of statistical distribution could be mistaken for a treatment effect, unless there is a control group in which the same regression to the mean would be observed. In uncontrolled trials, improvements that actually occurred due to the natural history of the disease could be incorrectly interpreted as evidence that the intervention was effective. Having a control group ensures that this mistake is not made, because the same improvement would occur in the control group, and there would be no difference between the control and active groups.

Despite the vital necessity of having a control group, it is a complex task to establish a control arm in the case of allergen-reduction trials, because there is such a vast plethora of potential factors that play a causal role in the severity of asthma symptoms. A very large control group would be required, to ensure that all the competing hypotheses are taken account of. It is an onerous task to measure each of the potential causal factors and outcome factors (see Table 1). A very large sample size is required to guarantee that such a large array of factors will be evenly distributed between the two groups after

randomisation. The study must be designed in such a way that reporting bias can be assessed between the active and control groups.

The mechanics of putting randomisation into practice are not simple. Concealment of random allocation requires that:

1. Staff who make the randomisation list, and who have access to it, are different from the staff who interact with subjects or analyse the data
2. Staff who enrol subjects do not know the allocation of the next slot in the randomisation list
3. Staff who enrol subjects cannot alter the order in which they assign subjects to slots in the randomisation list
4. Staff who make up the packets of active and control interventions, are not the same staff who interact with subjects or analyse data; and they make the packets up so there is nothing about the outward appearance of the packets that betrays what is inside them. The staff who do interact with the subjects, can take appropriately labelled intervention packets off the shelf and give them to the subjects without being aware of what kind of intervention the subjects received.

The complexity involved in having a control group explains why a number of studies have not had a control group. However, this limits the ability of those studies to adequately assess the intervention. Indeed, early trials of allergen-reduction were of poor methodological quality, and gave false optimism that this was a path worth exploring.

The early trials were conducted at a time where the scientific quality of similar environmental interventions to improve health was fairly poor.

The issues surrounding the scientific method for proving that there is a causal association between allergen-reduction and asthma severity, may be made clearer by corollary with the attempts to prove that there is a causal association between air ionisers and improved asthma (see Appendix 1)

If future studies of allergen reduction are to be able to truly test their hypotheses then they must use methods that are different to those that have been used to date, indeed the Cochrane reviewers explicitly state this in regard to HDM reduction studies [160]. Just like the air ionisation field of research had its final definitive trial performed by Nogrady & Furness (see Appendix 1), so too the field of allergen-reduction needs a trial (or series of trials) with a thoroughly rational logical study design, which can bring this field of enquiry to a similarly firm conclusion. This thesis describes what structure and methods such a trial would require, which will be an ambitious undertaking, and represent a sea-change in research.

### ***11.3 Appendix 3: Sources of alternative explanations to the one under investigation***

#### **11.3.1.1 Bias**

Bias occurs when some aspect of a study's design or conduct, results in the incorrect rejection or confirmation of the hypothesis being tested. For example, the use of acaricides (which are toxic to HDMs and/or denature the mite allergen) in carpets can potentially bias a study toward the conclusion that the application of acaricides does kill HDMs and denature the allergens, because any reduction in allergen concentration in carpets after the application of the acaricide may in fact just represent a dilution of the allergens by the acaricide, but not the destruction of the mites or their allergens. The only way to overcome this is by applying a placebo acaricide of equal volume to the carpet within the control group.

Deficiencies in study design, such as inadequate blinding, may result in a particularly strong bias in community-based allergen-reduction studies because they have a substantial subjective component. Ratings of asthma symptoms and quality of life are highly subjective, and even seemingly objective outcome measures like Peak Expiratory Flow are affected by subjectivity since the amount of effort that participants put into blowing into the meter, depends on their subjective state of mind. Therefore it is vital that the subjects in these studies are blind to which group they are in. Indeed, a meta-analysis of meta-analyses concluded that weaknesses in allocation concealment and lack

of blinding produced a marked bias in trials with subjective outcomes but had little effect in trials with highly objective outcome measures [201]. Alarming, these authors' meta-analysis suggests that among trials with subjective outcomes, failings in allocation concealment can exaggerate the results by 0.69 times, and defects in blinding can inflate the results by 0.75 times. This should caution any reader or researcher to pay strict attention to rigorous study methodology, not as a theoretical nicety, but a necessity.

### **11.3.1.2 Selection bias and allocation concealment**

Selection bias occurs when subjects get allocated to one or the other arm of the study due to a characteristic of the subjects. This will bias the study because the two groups will not differ solely by whether or not they receive the intervention, but instead will also differ by the factor(s) which caused them to be put into one group more often than the other. For example, if the allocation process of a community-based trial is not completely concealed, then researchers may assign children differentially to the active or control group on the basis of perceived need, so that those in higher need (severe asthmatics) get assigned to the active group, and those with less need (mild asthmatics) get assigned to the control group.

Selection bias is overcome by allocation concealment, which is the method of ensuring that researchers (or subjects) cannot choose which group subjects will be assigned to, and that instead the allocation does indeed occur as a result of randomisation. Allocation concealment can be done a number of ways, for example, by computer random number

generation, or by generation of a series of tamper-proof envelopes that contain allocation numbers.

As mentioned earlier, a study by Lesley Woods et al conducted a meta-analysis of a number of meta-analyses to assess the degree to which inadequate allocation concealment biased a study, and whether this was influenced by the nature of the intervention or outcome being studied [201]. The authors concluded that studies which had subjective outcomes were much more prone to exaggerate the association being measured by an odds ratio of 0.69 (95%CI 0.59 to 0.82) as a result of selection bias from lack of allocation concealment compared to studies with objective outcomes. On the other hand, for studies with objective outcomes, low quality blinding did not bias the outcome odds ratio 0.91 (0.80 to 1.03) [201]. While some meta-analyses found that trials with inadequate allocation concealment tended to exaggerate estimates of the intervention effect, others did not, and hence it required a meta-analysis of these meta-analyses to deliver some certainty, which underscores the magnitude of the sample size and the complexity of the design which is an absolutely necessary requirement if a trial is to be capable of answering these questions [201].

High quality allocation concealment in community-based allergen-reduction studies, requires a high degree of staff training, and a large number of staff members, which may stretch the resources of publicly funded research programmes.

### **11.3.1.3 Bias and blinding the researchers and participants**

Ideally blinding must occur at both the level of the subject and the researchers.

If a Researcher knows which group a subject has been allocated to, then the Researcher can bias the study in a number of ways. First, the Researcher may bias the study by providing a differential service to subjects in one group compared to the others. For example, a researcher may feel sorry for the subjects who are only getting the placebo, and might therefore give them more counselling than the active group, which would bias the study towards the null. Second, researchers may bias the study by recording positive and negative outcomes differentially between groups (for example, a researcher may be suspicious of a generic asthma medication and bias a dose-equivalence study by questioning and examining subjects for adverse events who are taking the generic drug more thoroughly than they do for subjects taking the original drug. Third, the researchers' prior beliefs about the interventions may cause them to explain the use of the intervention to the subjects with more of an optimistic tone to subjects in one group than the other. Fourth, at the conclusion of the trial researchers who are not blinded, may choose an analysis plan which favours the intervention in one group over the other.

If subjects' are aware of whether they are in the active or control group, then their prior beliefs about the interventions may have an effect on their health, their compliance with the protocol, and their recording of their results. This is overcome by blinding the subjects to their assignment, however in the realm of allergen-reduction this is difficult to achieve, for reasons that are elaborated further in the section "Placebo" below.

In the meta-analyses mentioned above by Lesley Wood et al, inadequacies in blinding produced slightly less bias than poor allocation concealment, at an odds ratio of 0.75 (0.61 to 0.93). Just as with low quality allocation concealment, ineffective blinding produced a greater bias in studies with subjective outcomes than those with objective outcomes, in which the bias has an odds ratio of 1.01 (95%CI 0.92 to 1.10) [201].

#### **11.3.1.4 Optimism bias**

Optimism bias is the unwarranted belief that a therapy (particularly a new therapy) will be effective [202]. The outcome measures of allergen-reduction trials such as symptom scores, and quality-of-life scores, are highly susceptible to optimism bias because they are extremely subjective outcomes measures, which are totally reliant on self-report, and cannot be biochemically validated. One of the most effective ways to account for the ‘optimists’ is to use a double-blind, placebo controlled design, in which the allocation of subjects is randomised, to attempt to have ‘optimism’ equally distributed between the two groups. In order for subjects in the control group to have something to be optimistic about, they should be given a placebo, yet many allergen-reduction studies do not give a placebo to their control group (see the “Placebo” section below)..

#### **11.3.1.5 Social desirability bias**

Social desirability bias is the skewing of a study’s findings due to subjects acting in a way that they think increases their esteem among their peers and others around them

(such as the researcher interviewing them). Typically social desirability bias involves subjects over-reporting desirable things (good asthma symptoms, absence of rodents, high compliance with cleaning procedures), and under-reporting undesirable things (poor asthma-related quality of life, presence of mould, failure to comply with cleaning procedures). Allergen-reduction trials are especially vulnerable to social desirability bias because many of the interventions involve cleanliness and hygiene which have important moral judgements associated with them, and just as questionnaires about other judgement-laden behaviours (such as condom use, illicit drug use, abortion) are affected by social desirability bias, so too are questionnaires about cleaning and compliance with hygiene interventions in allergen-reduction trials [203]. Many of the outcomes that can be skewed by social desirability bias in the field of allergen-reduction are not readily verified by objective measures.

#### **11.3.1.6 Recall bias and Reporting bias**

Recall bias is when subjects in one group remember things that are relevant to the study outcomes differentially between the groups. Reporting bias is when subjects in one group record their outcome data differently compared to subjects in the other group, for example if the subjects in the active group report positive findings more thoroughly and side-effects less thoroughly because they are so optimistic about the intervention they received. These forms of bias are overcome by the use of a placebo.

### **11.3.1.7 Bias by subject's desire to personally gain from the study**

In allergen-reduction studies, subjects often receive expensive and desirable implements and tools to reduce allergens, such as Highly Efficient Particulate Arrestance vacuum cleaners, high quality bedding, and intensive visits to medical clinics. This can lead to bias if subjects believe that their receipt of desirable interventions, and continued participation in the study, is dependent on what answers they give to questionnaires and what numbers they record on peak flow charts and symptom scores. This is a challenge not only for allergen-reduction studies, but for many other studies of valuable medical interventions, such as fertility treatment [204].

### **11.3.1.8 Instrument Bias**

The method of collecting dust samples to measure allergen levels can bias an allergen-reduction study. There are numerous ways in which dust collection can vary: the size of the area vacuumed, the site of the area vacuumed, and the power of the suction that the vacuum cleaner [205]. It is important to standardise the method of dust collection, and also to measure allergens concentration in dust rather than the absolute amount of allergen, since the absolute amount of allergen will vary with the variability in suction power of vacuum cleaners, and this will make it impossible to compare the results of studies conducted by different researchers [26].

The Enzyme Linked Immunosorbent Assay (ELISA) test to measure allergen levels, can be biased by the presence of chemicals in the carpet of subject's homes. For example, if subject's use carpet freshener then ingredients in the carpet freshener can affect the

enzymes in the ELISA which reduces its ability to detect HDM allergens [206]. Even more importantly, ingredients in acaricidal sprays such as tannic acid can also interfere with ELISA tests and result in overestimation of their allergen-reduction [207].

### **11.3.2 Confounding factors**

A confounding factor is a variable which is statistically associated with both the intervention and the outcome measure of a study, but is not on the causal pathway between the intervention and outcome. For a study to determine if a statistical association between the intervention and outcome is a causal one, or if the intervention is a confounding factor and the real cause is some yet unidentified factor, it is necessary to use an appropriate study design.

The randomised controlled trial (RCT) is considered to be the gold standard for research that aims to test putative causal associations because the RCT possesses a number of important study design features. The presence of a control group, and the random allocation of subjects between groups makes it very likely that confounding variables are equally distributed between the two groups (as long as the two groups have enough subjects in them) so that there will be no statistical difference in the prevalence of the potential confounder in one group compared to the other.

For allergen-reduction trials there is almost no limit to the number of potential confounding factors. The web of causality in the immune system and numerous endogenous chemical mediators of bronchoconstriction ensure that there are numerous suspects for what could be the cause of worsened asthma (see Table 2.1). In order to

decide whether the observed effect was due to confounding, a study must record sufficient details about the subjects, including their: environment, kind of asthma, atopic status, allergen-levels, social stress, asthma medication, air pollution, outdoor and indoor temperature, to name but a few (see Table 2.1).

To reliably rule out confounding factors as the cause of improved asthma, allergen-reduction studies must be extremely complex. The Morgan and colleagues study was part of an extremely large multi-centre multi-study-design trial across the United States of America, which was capable of examining the role of confounding factors. The various studies that made up this large programme, were all published as separate papers, which makes it difficult to put the outcomes of all the studies together to assess the role of confounding and whether allergen-reduction works. The present thesis has attempted to make this overall gestalt assessment across the studies that were identified in a systematic review of the literature, rather than confining the analysis to the Morgan and colleagues study and the other studies within the Inner City Asthma Study.

### **11.3.3 The Hawthorne Effect**

The Hawthorne Effect is a change that occurs in people's behaviour as a result of them being observed, and which is not due to the intervention that is being studied. The notion of the Hawthorne Effect was developed in 1955 by Henry A. Landsberger who investigated whether employee productivity could be increased by altering the level of lighting at the Hawthorne Works. He found that productivity improved for a short but limited period after both increasing the lighting and also decreasing the lighting.

Landsberger concluded that the short-term improvement in productivity was not due to the lighting, but due to the fact that the employees were being observed, and therefore they felt their bosses were taking an interest in their wellbeing. There are numerous instances in both social science and physical science when the act of observing a phenomenon alters the phenomenon, and hence creative methods must be devised in order to detect and examine the phenomenon further. For example, in order to visually “see” something, it is necessary that light waves bounce off it and then hit the retina of the eye, or a photographic plate. However, in order to observe an electron for example, the collision of light rays on the electron would destroy it, and hence electrons cannot be observed visually, and their existence has to be inferred indirectly.

The Hawthorne Effect can be mistaken for the treatment effect in uncontrolled trials, and can bias a controlled study toward confirming the null hypothesis control group know they are being observed and receive the same amount of ‘observation’ as the active group. The only way to fully overcome the Hawthorne Effect would be to have an arm of the study in which the subjects do not know they are being studied and the researchers have no interaction with the subjects. However, by having no contact with researchers, the subjects in such a group are likely to receive less than the usual standard of medical care, which would be unethical. However, the delivery of basic standard care to the control group, would involve a lot of contact and observation with researchers (especially for severe asthmatics), and hence it would be difficult to avoid the possibility that the Hawthorne Effect may play a role.

The Hawthorne Effect was investigated in two asthma intervention studies, which found some evidence of it acting within the minds of the asthmatic patients (or their parents), and also within doctors themselves.

A study by Greineder and colleagues tested whether the simple fact that a child received a referral to an asthma outreach program caused them to reduce their healthcare utilisation (the Hawthorne Effect), or whether the cause was indeed the asthma outreach program. Greineder and colleagues compared the healthcare utilisation in children who received the referral and also attended the outreach program, with children who received the referral but did not attend. There was a substantial but statistically non-significant reduction in utilisation among children who received the referral but did not attend, and while this may be a chance finding, the authors suggests that the receipt of a referral to a programme may have a Hawthorne Effect [208]. When the improvement in the control group was subtracted from the improvement in the active group, the remaining effect size (which could be attributable to the education and allergen-reduction advice) was greatly reduced, but still statistically significant: 60% reduction in emergency department visits, 74% hospitalisations, and 72% reduction in community healthcare in the active group compared to control group. Although the differences between groups were statistically significant, the operation of the Hawthorne Effect (or perhaps, more accurately, the Pygmalian Effect) had a substantial impact on the economic analysis with the cost-savings estimate reduced from \$11.69 per dollar spend on intervention to \$6.49. If this study had not had a control group, it would have over-estimated the effect of the interventions.

In a study of whether more intensive prescription of corticosteroids to paediatric asthma patients at their initial presentation of acute asthma exacerbations to the Emergency Department, reduces their risk of representing within the following week [209]. The rate of re-presentation with asthma within one week was compared between 1. a retrospective audit and a subsequent prospective audit, and 2. between the enrolled and non-enrolled subjects in the prospective audit.. No informed consent was obtained for the retrospective audit, whereas subjects in the prospective study were asked for consent (those who consented were enrolled, those who did not consent were not enrolled but their readmission rates were available). The rate of repeat visits was greater during the initial retrospective period ( $68/526 = 13\%$ ) than the prospective period ( $57/725 = 8\%$ ). However, among the prospective subjects, there was no statistically significant difference in the rates of repeat visits between those who consented and were enrolled (and therefore could be considered to be under observation) and those who did not consent and were not enrolled (and therefore would not have felt that they were being observed). The absence of any difference in readmission rates in the enrolled and non-enrolled subjects indicates that the intensive corticosteroids did not prevent readmissions, and that the subjects did not experience the Hawthorne Effect in their own minds. Yet despite the lack of evidence of any effect of the intervention in the prospective study, nonetheless there was a statistically significant decrease in rates of re-presentation to the ED, and hospital admissions, between the retrospective audit period and the subsequent prospective phase, which was independent of whether or not subjects were enrolled or not. Since the intervention cannot explain this change in healthcare utilization, the next most plausible

explanation is that the Hawthorne Effect was operating within the minds of the ED physicians who may have changed their prescribing of steroids because they knew they were being audited. However, the patient notes were incomplete, and so it was not possible to actually confirm whether clinical practice had been altered by the Hawthorne Effect.

#### **11.3.4 The Pygmalion Effect**

The Pygmalion Effect is the improvement in a person's ability to perform a task due to his or her knowledge that other people have a high degree of confidence in his or her ability to perform it. Allergen-reduction studies are prone to the Pygmalion Effect because subjects may improve their compliance with asthma management plans as a result of the high expectations they believe the researchers have of them, and hence the subject's asthma improves due to the researcher's high expectations, and is not entirely due to the allergen-reduction intervention. In a study when the researchers are not blind to the study allocation, this can bias the study toward falsely rejecting the null hypothesis because the subjects in the active group will experience higher expectations than those in the control group since it is likely that the non-blinded researcher will not be able to hide his or her greater confidence in the active subjects compared to the control subjects. If researchers are blind, and do treat both groups with equal confidence in their ability to comply with the study and improve their asthma, then the Pygmalion effect may lead to improved asthma in both groups, which will tend to obscure any benefit from the allergen-reduction in the active group. If there was not a control group, and the

improvement in the asthma of the active group is solely due to the Pygmalion Effect, then the improvement could be incorrectly interpreted as being due to the allergen-reduction.

An allergen reduction intervention can only be found to be effective if 1, the trial has a control group, 2. the control group gets the same quality of interaction and empowering interaction with researchers as the active group (in other words there is a placebo), and 3. if the effect of the allergen-reduction is larger than the improvement in the control group caused by the Pygmalion Effect.

The Hawthorne Effect and Pygmalion Effect are particularly relevant to allergen-reduction studies, since they involve subjects being actively observed, and given attention and benefits from researchers who clearly have an interest in their well-being and have an optimism that their health can be improved. The Hawthorne and Pygmalion Effects, not the allergen-reduction interventions, may be responsible for the improvements in early non-randomised non-placebo-controlled trials that gave rise to the notion that allergen reduction may improve asthma. For example, improved asthma from relocating asthmatics to high altitude in 1924 may have been due to the Hawthorne Effect rather than the low HDM allergens in the Alps, particularly since the improvements in their asthma were only temporary, just as the improvements in staff morale in the Hawthorne Factory were only temporary [210].

### **11.3.5 Blinding**

Blinding of subjects is necessary to ensure their improved outcomes are not simply due to their belief and expectation that they are in the active group and are therefore likely to be receiving something efficacious. The degree of blinding that is required is debatable. Some researchers would argue that subjects should be completely blind as to which group they are allocated to. Others would argue that it does not matter that the subjects know what group they are in, as long as they are unable to decide whether the group is the active or control group (for example, some would say it is not a problem if subjects know they are in the mattress encasings group whereas others are receiving a dehumidifier instead of encasings, as long as they do not know which intervention is hypothesised to be active). Blinding can be taken to an extreme, however, and some would even argue that ideally subjects should even be blind to whether they are part of an experiment [75], however it would be hard to conduct such a study without contravening subjects' human rights and the fundamentals of Ethics. Clearly, some more mild form of blinding is necessary in the real world.

An example of how an open-label uncontrolled study can find a positive association, but when a randomised, blinded, placebo-controlled study is done subsequently, no association is found is the study of natamycin spray to kill HDMs. The early study that had a low-quality study design (open-label, non-random, non controlled, small sample size) produced very encouraging results. Just over half the subjects (54%, 15/28) were improved, nearly a third (32%, 9/28) remained unchanged, and only a minority (14%, 4/28) got worse after using natamycin spray [211]. However when the natamycin

hypothesis was retested using a rigorous study design, no improvement in asthma was observed [102]. The caveats that must be placed on the positive findings of these small low-quality studies are justified by the subsequent clear demonstration in studies of robust design that the early optimism was unfounded.

Ideally a study would have a factorial design in which there are several control groups: one that is given a placebo to account for the placebo effect, one that is just observed but given no placebo, and one that is given no placebo and is not observed until the end. The Hawthorne Effect and Pygmalian Effect can be assessed by comparing the later two groups.

Group 1: R O<sub>1</sub> X O<sub>2</sub>

Group 2: R O<sub>3</sub> O<sub>4</sub>

Group 3: R X O<sub>5</sub>

Group 4: R O<sub>6</sub>

### **11.3.6 Placebo**

Approximately one third of subjects experience a positive effect from a placebo intervention when its effects are rated subjectively by the subjects [212]. Not only can the placebo effect give rise to subjective responses, but it can result in physical changes as well [213]. A study demonstrated that if asthmatics are given normal saline aerosol that they are told will give them an asthma attack, a proportion of them (19 out of 40)

will indeed have an attack, and this attack will resolve when they are given the same aerosol but told that it would cure their attack [214]. In order to distinguish a real effect of the intervention from a placebo effect, it is necessary to take proper account of the placebo effect through the design of the study. The placebo effect occurs due to the perceptions that subjects have of the procedure, and there a number of processes that influence subjects' perceptions, including the appearance of the placebo, and the manner in which the researchers explain and deliver the placebo. In an ideal clinical trial the placebo effect may be minimised by limiting these mechanisms by restricting the amount of information that subjects have about the interventions and the contact time between subjects and researchers. However this is not feasible in trials that intrinsically require substantial contact time to be spent between researchers and subjects such as in psychotherapy or educating subjects on cleaning interventions for allergen-reduction [215].

A more effective method of managing the placebo effect than restricting information and contact with researchers, is to have a control group, which should receive what appears superficially to be exactly the same treatment as is given to the active group, and therefore if this active group intervention produces a placebo effect, this should be seen in the control group as well as the active group, so that when the two groups are compared if there is any difference in outcome between the two groups it cannot be due to the placebo effect.

The advantages of having a placebo for the control group are two-fold: first, it should give the control group the same psychological effect as the active group got from the active intervention, and second it maintains blinding to study allocation if it is equivalent in appearance to the active intervention.

A placebo needs to be sufficiently believable to give the control group subjects confidence in it, but not produce a greater Hawthorn or Pygmalion effect than that which is produced by the active intervention. It is important to have a placebo which has a neutral effect on the outcomes of interest. If a placebo intervention has a positive impact of improving asthma, then it will bias the study towards incorrectly accepting the null hypothesis. In allergen-reduction research, the placebo interventions in the control group will inevitably have some positive effect on asthma because, firstly, it is technically difficult to devise convincing but inactive placebo allergen-avoidance interventions, and secondly, the contact between researchers and control group subjects will inevitably provide a Pygmalion effect that cannot be minimised because researchers must spend time with control group subjects to explain the placebo interventions and collect the outcome data.

It is hard to come up with behavioural placebos and educational placebos. It is not readily obvious how a researcher can advise people to do a complex behaviour that the researcher knows is ineffectual. There is a fine line between telling an “inventive story”, and telling a lie. The existence of a placebo may make it harder to recruit subjects to enrol in the trial, because they may not be happy to know that they have a 50% chance of

getting a sham intervention, and will end up expending energy and effort carrying out procedures that never had any chance of being effective.

The size of the impact of the placebo-effect on biasing a study's outcome is not entirely clear, and is an issue that has been debated by Cochrane reviewers and others [216-220]. Wampold et al assert that there is evidence from systematically comparing placebo-controlled trials to non-placebo controlled trials, that there is a substantial placebo effect for most kinds of placebo interventions. Hrobjartsson & Gøtzsche argue that in fact the opposite is true, and that their review of the literature, which examined more papers than Wampold and colleagues, showed no evidence for a notable placebo effect [216, 220]. Hrobjartsson & Gøtzsche note that the trials that seem to support the existence of a placebo effect, were smaller trials with continuous outcomes, and what might look like the placebo effect was in fact simply sample-size bias, and in studies that were not blind was likely to be reporting bias not the placebo effect. Wampold et al [219] counter Hrobjartsson's and Gøtzsche's assertion that there is no placebo effect quite convincingly, by pointing out that:

1. Hrobjartsson & Gøtzsche compared studies on the basis of the condition being treated, rather than on whether that condition might be influenced by the placebo-effect. The placebo effect is contextually based – it differs depending on what kind of condition that is being examined, for instance it is plausible to hypothesize that pain might respond to the placebo-effect whereas it is not plausible that cancer would [219]. .

2. Hrobjartsson & Gøtzsche assumed that all studies which were not double-blinded inflated the placebo-effect, whereas Wampold et al examined each study on a case-by-case basis to determine how much it might be amenable to the placebo-effect.
3. Wampold et al argue that Hrobjartsson & Gøtzsche did not take into account the context in which the placebo interventions were delivered, which would have a considerable bearing on how convincing the placebos were. The manner and location in which researchers give the explanation to subjects, is as important, if not more important, than the placebo device itself, in producing a placebo effect in the subjects. Not all placebo interventions are created equal, and how effective they are depends on the context in which they are deployed.

One can conclude from this debate that Hrobjartsson & Gøtzsche have put too much emphasis on including greater numbers of trials in their meta-analysis. Although their inclusion of a greater number of studies does reduce the bias that can occur from selecting only a few of the available trials, this comes at the cost of 1. not adequately dealing with the heterogeneity in the trials they included, and 2. not being guided by theory and biological plausibility in choosing which studies to include and how to group them for analysis. Even if Hrobjartsson and Gøtzsche are correct, and the improvement in placebo-controlled groups compared to non-placebo-controlled groups is not the result of a placebo effect but was caused by reporting bias, this does not reduce the value of having a placebo in the control group, for it is just as important to remove reporting bias from a study as it is to remove the placebo effect.

The plausibility and efficacy of a placebo depends more on the words used by the researcher to create expectations about the placebo than on any features of the placebo itself. The power of the style in which a placebo treatment is described, is illustrated by a study of 200 patients who may have had psychosomatic symptoms. In this study subjects were given either a positively delivered medical consultations with or without treatment, or medical consultation delivered in a negative manner with or without treatment. 64% of subjects felt better when they received a positive medical consultation, compared with 39% of those who received a negative consultation ( $p = 0.001$ ), and the improvement cannot have been due to the treatment intervention because 53% of those treated felt better compared with 50% of those not treated ( $p = 0.5$ ) [221]. Clearly the subjects who felt better must have experienced the placebo effect, because whether they felt better did not depend on whether they received the intervention but whether they received a positive consultation, and clearly the causal agent that gave rise to the placebo effect was the manner in which it was presented to the subjects, not the intervention itself.

It is not possible to conclude that a study was not subject to the placebo effect on the grounds that not only did the subjective measure improve, but the objective measures improved as well. There is evidence that the placebo effect not only impacts on subjective states of mind, but also physically alters neurochemical pathways [222], such as the opioid receptors that mediate pain perception [223, 224], and the neural circuitry that modulates the immune system [225-230]. The placebo effect has been observed with studies of asthma [213, 231]. Of particular interest for allergen-reduction, is the finding that the placebo effect is able to reduce not only subjective symptom, but also objective

measures of bronchoconstriction and broncoprovocation [213], and the biochemical pathways involved in allergic rhinitis [232]. Indeed, placebo treatment reduced the deterioration in PEF compared to control treatment, in children with exercise-induced asthma, although not by quite as much as treatment with salbutamol [231]. Goebel et al showed that human subjects who were allergic to HDMs could be conditioned to produce a symptomatic response, reduced skin prick test and basophil activation with exposure to a placebo beverage that looked identical to a beverage containing the histamine antagonist desloratadine. This physical manifestation of the placebo effect in the activity of the immune system is analogous with how emotions also have a physical impact on the immune system and allergic responses .

A placebo is not only needed to account for the psychological placebo effect, it can sometimes be necessary for practical physical reasons. A non-psychological example of why an appropriate placebo is needed for the control group, is that if the active group receives an acaricide powder to apply to their carpet, and there is no placebo acaricide powder for control group, then any reduction in the concentration of carpet allergen levels in the active group may simply be due to dilution by the acaricide, and hence is not definite evidence for the acaricidal or allergen-denaturing power of the putative acaricide.

One of the main reasons for conducting this thesis was to determine if a placebo could be devised which was practical in the New Zealand domestic environment, in order to determine if the improvements in asthma seen in the Morgan and colleagues study were simply the result of the placebo effect.

### **11.3.7 Multiple comparisons**

There is a considerable array of asthma outcome measures, which gives rise to the need to adjust for multiple comparisons when calculating statistical significance, and the need to be aware of selective reporting of positive results within trials. Trials of new therapeutic methods may report the outcome measures that improved with the therapy, but not report those that did not improve, which can lead to an optimism bias about that therapy [202].

### **11.3.8 Non-refutable arguments**

Some proponents of the allergen-reduction hypothesis accept the findings of studies that provide evidence for the hypothesis, but reject the findings of studies that find no evidence for the hypothesis. They justify this selective interpretation of the literature by arguing that the reason the negative studies found no evidence was because they had flaws in their designs. For example, it is said that the negative studies were too small, did not enrol the right subjects, did not have an effective enough intervention, did not provide the intervention for a sufficient duration of time, did not provide interventions against multiple allergens, or failed to enrol sufficiently severe asthmatics [89, 233],

This is a risky argument because it is logically flawed. It is a non-refutable argument: one can always counter a negative finding with the retort “but you could have lowered more kinds of allergens, by a larger amount of reduction, and for a longer period of time”. The early educationalists in their call for the relaxation of strict experimental design rules, to allow for research in real-world settings, were aware of the danger of the many ‘get out of jail’ cards that irrefutable arguments have: “. . . many hypothesis-sets are so double-jointed that they cannot be disconfirmed by available probes.” [75]. This is an especially troublesome issue for allergen-reduction research, because there are so many potential causal factors, that there are a seemingly infinite array of reasons why a study may have failed to provide evidence to support the argument that allergen-reduction leads to improved asthma control.

A number of authors utilise irrefutable arguments, despite their inherent risk of jeopardising the truthfulness of the argument’s conclusions. For example, Richter [130] criticised the study by Kinney et al [97], for failing to undertake a sufficiently comprehensive range of interventions, to address the ecological problems that are systemic to low socioeconomic housing in inner-city areas in the United States, and argues that if they had used more effective interventions they would have found an improvement in asthma.

## 12 Footnotes

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<sup>I</sup> “Unexpectedly, the placebo air-cleaners also captured dust and allergens because of the presence of a coarse endfilter, which was added in order to prevent unblinding of patients and investigators. Eight of these end-filters of the placebo air-cleaners were available for analysis; the amount of dust captured in end-filters from air-cleaners in living-rooms was 3.17 g (mean), and in bedrooms was 2.12 g.” page 1221 of 104. Van der Heide, S., et al., *Allergen reduction measures in houses of allergic asthmatic patients: Effects of air-cleaners and allergen-impermeable mattress covers*. European Respiratory Journal, 1997. **10(6)**: p. 1217-1223.

<sup>II</sup> One might think that the reduction at 2 weeks, increase at 6 weeks, and reduction at 24 weeks represents an overall ‘trend’ for reduction. However, one cannot be confident that a trend exists, unless one first performs statistical tests, to determine whether there is a significant trend. There are a range of formal statistical tests for trend, including the chi-squared test, Pearson’s correlation coefficient, and Poisson regression analysis.

<sup>III</sup> While, it is biologically plausible that, at the level of an individual patient at a discrete point in time, the peak flow might not improve, but their symptoms could improve; this is unlikely to occur consistently in outcome data averaged across numerous different subjects in a trial with a large sample size. It is possible that one person’s symptoms could improve but their peak flow does not, but it is unlikely that this will happen consistently on average for the whole sample. It is more likely that there will be congruence between objective asthma outcomes compared to subjective ones (like symptoms). Consistency is a Bradford-Hill criterion, and the lack of consistency in these observations, lessens the likelihood of there being a causal association between the allergen-reduction and changes in asthma.

<sup>IV</sup> In Māori, ‘hoha’ means ‘angry’ or ‘bored’.

<sup>V</sup> The SPT identifies people who are capable of a cellular immunologically mediated allergic response to allergens; whereas an inhalation test should be capable of identifying people who are capable of reacting in a ‘direct chemical irritation’ mechanism (such as the enzymatic proteolytic action of Derp1).