

# **MOUSE ALLERGEN AND ASTHMA INTERVENTION TRIAL (MAAIT)**

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## Statement of Compliance

The Mouse Allergen and Asthma Intervention Trial (MAAIT) will be conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), and the following applicable regulatory requirements:

- The trial will be conducted in compliance with U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46 and 21 CFR including parts 50 and 56 concerning informed consent and IRB regulations, if under IND, 21 CFR 312).
- Completion of Human Participants Protection Training will be required of all key study personnel in compliance with NIH policy.
- The trial will be conducted in compliance with NIAID Clinical Terms of Award.

## Signature Page 1

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

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## List of Abbreviations

*This list should be modified to include protocol-specific terms.*

AAs	African Americans
AE	Adverse Event/Adverse Experience
ACT	Asthma Control Test
ATAQ	Asthma Therapy Assessment Questionnaire
BD	Below detection
Bla g 2	Cockroach allergen
CAB	Community Advisory Board
Can f 1	Dog allergen
CAP-RAST	CAP-radioallergosorbent test
CCAC	Clinical Coordinating and Administrative Core
CFR	Code of Federal Regulations
CHSA	Children's Health Survey for Asthma
CI	Confidence Interval
CMP	Clinical Monitoring Plan
CRF	Case Report Form
CRO	Contract Research Organization
CV	Clinic Visit
DAIT	Division of Allergy, Immunology, and Transplantation
DMAC	Data Management and Analysis Core
EBSC	Environmental and Biologic Sample Core
ED	Emergency Department
ELISA	Enzyme-linked Immunosorbent Assay
eNO	Exhaled Nitric Oxide
FDA	Food and Drug Administration
Fel d 1	Cat allergen
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GCP	Good Clinical Practice
HV	Home Visit
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug
INR	International Normalized Ratio
IPM	Integrated Pest Management
IRB	Institutional Review Board
IRR	Incidence Rate Ratio

## List of Abbreviations

IT	Integrated Pest Management Technician
ITT	Intent to Treat
JHCP	Johns Hopkins Community Physicians
MAAIT	Mouse Allergen and Asthma Intervention Trial
MAACS	Mouse Allergen and Asthma Cohort Study
MOP	Manual of Procedures
N	Number (typically refers to participants)
NCICAS	National Cooperative Inner-city Asthma Study
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIEHS	National Institute of Environmental Health Sciences, NIH
NHLBI	National Heart, Lung, and Blood Institute, NIH
NIH	National Institutes of Health
Mus m 1	Mouse allergen
OCR	Optical Character Recognition
OHRP	Office for Human Research Protections
PEM	Personal Environmental Monitor
PFT	Pulmonary Function Test
PI	Principal Investigator
PM	Particulate matter. $PM_{10}=PM\leq 10\text{microns}$
PP	Per Protocol
QA	Quality Assurance
QC	Quality Control
RA	Research Assistant
RCT	Randomized Control Trial
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SPT	Skin Prick Test
TC	Telephone Call

## Protocol Summary

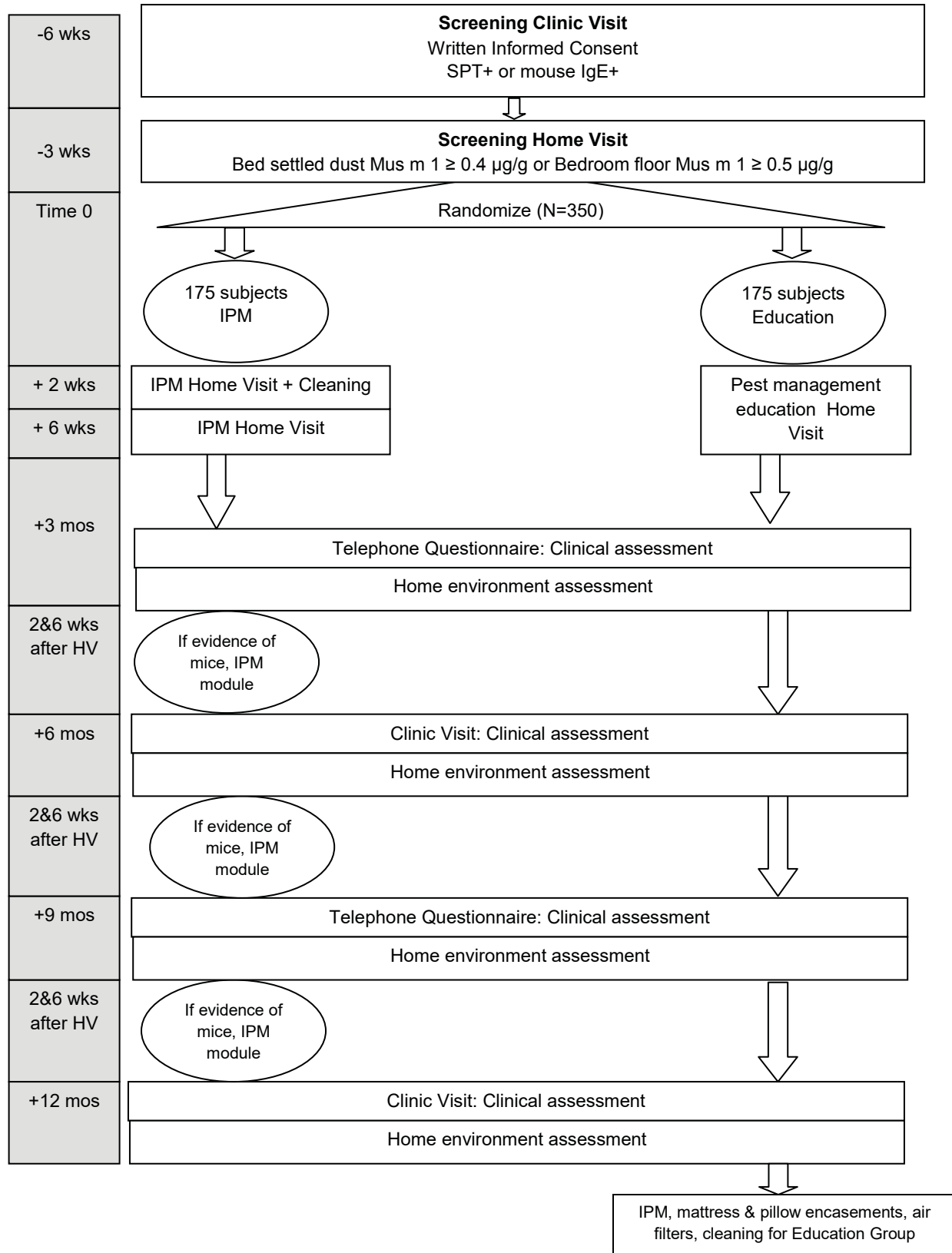
<b>Full Title</b>	Mouse Allergen and Asthma Intervention Trial
<b>Short Title</b>	MAAIT
<b>Clinical Trial Type</b>	Environmental Intervention Study
<b>Principal Investigator</b>	Elizabeth Matsui, MD MHS
<b>Sample Size</b>	350; as many as 125 from the Boston site and as many as 250 from the Baltimore site.
<b>Study Population</b>	5-17 year old children with moderate to severe asthma who live in homes with high levels of mouse allergen
<b>Accrual Period</b>	36 months
<b>Study Design</b>	<p>The study is a randomized, controlled trial of a mouse-targeted integrated pest management (IPM) intervention in childhood asthma. There will be two parallel arms, one arm will receive IPM (extermination and sealing of sealing of holes and cracks), education, cleaning, mattress/pillow encasements and room air filters (IPM Group) and the other arm, the Education Group, will receive pest management education alone (education regarding setting traps, sealing holes and cracks and housekeeping practices). The IPM Group will receive two IPM visits approximately 4 weeks apart after randomization and subsequent IPM visits will be driven by continuing or recurring evidence of mouse infestation. The Education Group will receive one intensive education visit within 6 weeks of randomization. Clinical outcomes will be assessed every three months and followed for a total of 12 months in both groups. Home environments will be assessed every three months for a total of 12 months in both groups. Mouse-specific IgG levels will be quantified at screening and 12 months as a biomarker of mouse allergen exposure. After finishing the study the Education Group will be offered IPM, cleaning, mattress/pillow encasements and room air filters at a visit that is to be scheduled within 2 months of their last study visit (either CV3 or HV5). This visit is not required if participants decline, do not schedule, or no show for the visit. Participants are given a letter explaining the</p>

	details of this IPM exit visit.
<b>Study Duration</b>	<b>There will be an approximately 6 week clinical and home assessment period to determine eligibility prior to randomization, and 12 months of study duration following randomization. The total duration will be approximately 13.5 months.</b>
<b>Study Agent/Intervention Description</b>	The IPM intervention will include placement of mouse traps, placement of rodenticide tracking powder in cracks and holes, sealing of holes and cracks with copper mesh and foam, cleaning, education, mattress and pillow encasements, and two room air filters. The first IPM module will consist of two IPM home visits, approximately 4 weeks apart. Subsequent IPM modules will consist of two IPM visits approximately 4 weeks apart and will occur if there is evidence of continuing or recurrent mouse infestation at the home assessment visits at approximately 3, 6, and 9 months.
<b>Primary Objectives and Outcomes</b>	<p>The primary objective is to determine the efficacy of this mouse allergen-targeted environmental intervention among mouse-sensitized children with asthma to improve asthma outcomes.</p> <p>The primary outcome measure is maximum days of symptoms/ 2 weeks. Maximum days of asthma symptoms will be assessed by questionnaire.</p> <p>Note:</p> <p>Maximum symptom days are defined as the highest of three specific symptoms variables that capture days or nights of symptoms over the preceding 2 weeks: (1) nights of waking, (2) days of slowed activity, and (3) days of wheezing, coughing or chest tightness. Thus, each subject has a value for the maximum symptoms days variable at each time point.</p>
<b>Secondary Outcomes</b>	Secondary outcomes for the mouse allergen exposure objective include: (1) bed, bedroom floor, and kitchen settled dust mouse allergen levels, (2) airborne mouse allergen levels, and (3) mouse-specific IgG and IgE levels as biomarkers of mouse allergen exposure. Secondary outcomes for the clinical objective include: (1) days of short-acting beta agonist use, (2) days of slowed activity due to asthma, (3) days of exercise-induced symptoms, (4) days of cough without an upper respiratory infection, (5) nights of waking due to asthma symptoms, and (6) number of hospitalizations, (7) emergency department visits, (8) unscheduled physician visits, and (9) prednisone bursts. These data will be obtained

	from questionnaires. Other secondary outcomes include FEV1/FVC, FEV1 percent predicted, percent change in FEV1 after short-acting beta agonist, and exhaled nitric oxide levels. These outcomes will be assessed using the Koko spirometer and Niox MINO.
<b>Rationale</b>	Mouse allergen exposure in urban communities is associated with asthma morbidity and integrated pest management (IPM) has been shown to reduce home mouse allergen levels by 75%. It is not clear whether a mouse allergen-targeted intervention can reduce asthma morbidity among mouse-sensitized children with asthma.
<b>Participating Sites</b>	Johns Hopkins University, Baltimore, MD  Boston Children's, Boston, MA  Columbia University, New York, NY (NOT a clinical site)
<b>Inclusion and Exclusion Criteria</b>	<p><b>Participant Inclusion Criteria</b></p> <ul style="list-style-type: none"> <li>• Males and females who are 5-17 years of age, inclusive, at the screening visit</li> <li>• Have physician-diagnosed asthma at least 1 year prior to the screening visit, or asthma symptoms for at least 1 year</li> <li>• Meet criteria for current persistent asthma defined as either: <ul style="list-style-type: none"> <li>(a) On a long-term controller medication for asthma, or</li> <li>(b) Meet NAEPP guideline requirements for persistent disease:(NAEPP expert panel report. Managing asthma during pregnancy: recommendations for pharmacologic treatment-2004 update)</li> </ul> </li> </ul> <ol style="list-style-type: none"> <li>1. Asthma symptoms 3 or more days per week over the past 2 weeks OR</li> <li>2. Nocturnal asthma symptoms at least 3 times in the past month</li> </ol> <ul style="list-style-type: none"> <li>• Have evidence of uncontrolled disease as defined by at least one of the following: <ol style="list-style-type: none"> <li>1. One asthma-related unscheduled visit to an emergency department (ED), clinic or urgent care facility in the previous 12 months</li> <li>2. One asthma-related overnight hospitalization in the previous 12 months</li> </ol> </li> </ul>

	<p>3. One or more bursts of oral corticosteroids in the previous 12 months</p> <ul style="list-style-type: none"> <li>Sensitized to mouse             <ol style="list-style-type: none"> <li>either a positive SPT (net wheal <math>\geq 3</math>mm) or</li> <li>a positive mouse-specific IgE tested, as quantified using the ImmunoCAP mouse urine reagents (<math>\geq 0.10</math> kU/L)</li> </ol> </li> <li>Have <math>\geq 0.40</math> <math>\mu\text{g/g}</math> of Mus m 1 in the bed settled dust sample or <math>\geq 0.50</math> <math>\mu\text{g/g}</math> of Mus m 1 in bedroom settled dust</li> <li>Reside within a geographic area of the study site so that home visits are feasible. In Baltimore, participants must reside within the beltway, in Boston, participants must reside within the Greater Boston area.</li> <li>Have no plans to move within the upcoming 12 months</li> </ul> <p><b>Participant Exclusion Criteria</b></p> <ul style="list-style-type: none"> <li>Pregnancy (determined by urine pregnancy test)</li> <li>Lung disease, other than asthma, that requires daily medication</li> <li>Cardiovascular disease that requires daily medication, excluding hypertension</li> <li>Taking a beta-blocker</li> <li>Currently taking Xolair</li> <li>Currently receiving Immunotherapy (allergy shots)</li> <li>Sleeping in another home 4 or more nights/week</li> <li>A positive urine screen for high levels of urine cotinine (<math>\geq 3</math> on the NicAlert) or self reported active smoker for children <math>\geq 12</math> years of age</li> <li>Unable to access areas of home necessary to conduct extermination</li> <li>Home in extensive state of disrepair as determined by Study Coordinator/PI judgment</li> </ul>
<b>Study Procedures</b>	<p><b>The IPM Group procedures will include:</b></p> <ul style="list-style-type: none"> <li>Targeted cleaning to remove allergen reservoirs</li> <li>Extermination (placement of traps and application of rodenticide)</li> <li>Pest management education (education regarding setting traps, sealing holes and cracks, and housekeeping practices (see MOP))</li> <li>Sealing of holes and cracks in the home</li> <li>Two room air filters will be placed in the participant's bedroom and TV/living room.</li> <li>Allergen-proof mattress and pillow encasements</li> </ul>

	<p><b>The Education Group procedures will include:</b></p> <ul style="list-style-type: none"> <li>• Pest management education (education regarding setting traps, sealing holes and cracks, and housekeeping practices (see MOP). <u>Upon completion of the study:</u> Extermination, sealing of holes and cracks, allergen-proof mattress and pillow encasements, cleaning and room air filters will be offered to subjects in this group after they finish the study.</li> </ul>
<b>Statistical Considerations</b>	<p>The MAAIT is a parallel group, randomized controlled trial designed to test the efficacy of an integrated pest management intervention on reducing household mouse allergen levels and improving asthma outcomes in mouse-sensitized children with asthma. Asthma symptoms, rescue medication use, and health care utilization will be compared between IPM and Education Groups to assess efficacy of the intervention in reducing asthma symptoms and morbidity.</p> <p>The primary clinical outcome measure, maximum number of days of symptoms in the preceding two weeks, will be assessed at the same time points as allergen levels (screening, 3, 6, 9, and 12 months) because this outcome variable has been shown to be related to current mouse allergen levels. Other clinical outcome variables that will be examined will include: days of rescue medication use, nights of nocturnal symptoms, days of exercise-related asthma symptoms, days of cough without a cold. These variables will be treated as count variables and analyzed accordingly. For key secondary outcome variables related to health care utilization that are rarer than symptom days, the mean number of each type of event will be analyzed; however, if these events are rare the variables will dichotomized. Prednisone bursts, unscheduled doctor visits, emergency department visits, and hospitalizations will also be examined.</p>

**Schematic of Study Design:**



# 1. KEY ROLES

## 1.1 Sponsor, Sites, and Investigators

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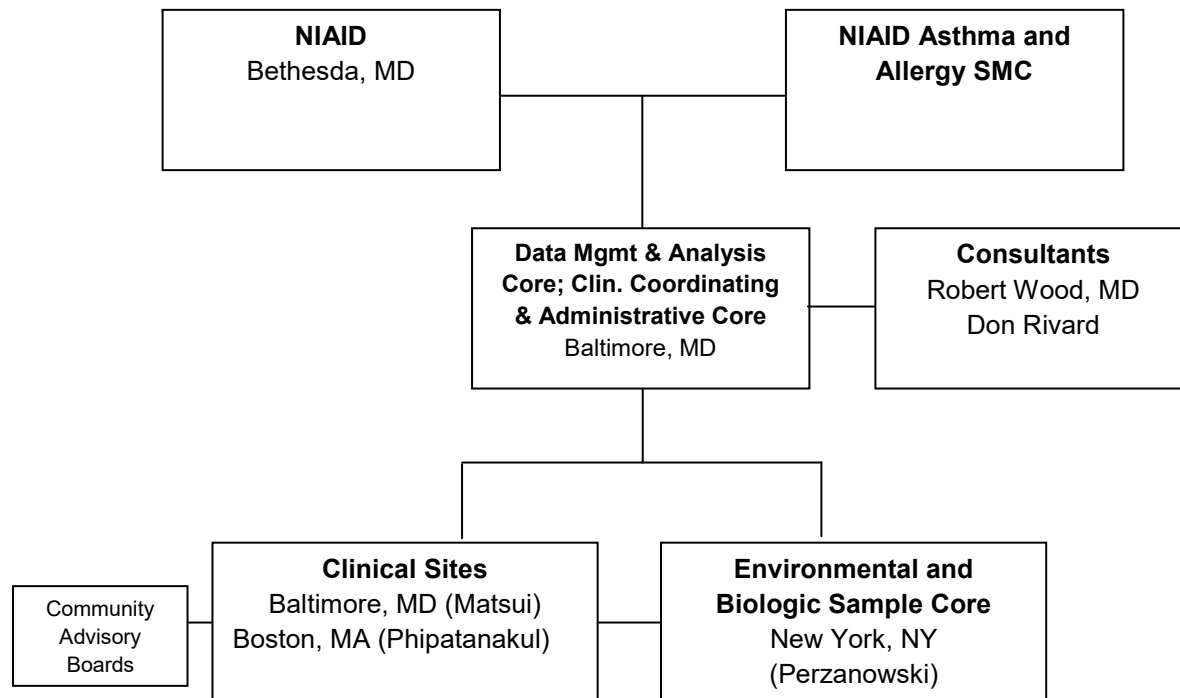
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## MAAIT Organizational Chart



## 2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

### 2.1 Background Information

There are striking differences in environmental exposures between urban homes and suburban homes that may, in part, explain the disproportionate asthma burden in the inner-city population.(Matsui et al.;Matsui et al.;Matsui et al.;Leaderer et al.) In urban homes, pest allergens, such as cockroach (*Blattella* sp.) and mouse (*Mus mus*), are the predominant indoor allergens.(Matsui et al.;Eggleston et al.;Phipatanakul et al.;Chew et al.;Chew, Correa, and Perzanowski;Phipatanakul et al.) Previous studies indicate that mouse allergen may be a critical factor in the inner-city asthma burden, particularly in the major metropolitan areas of the Northeastern United States.(Chew, Correa, and Perzanowski;Phipatanakul et al.) For example, *Mus mus* is found in 95% of inner-city homes,(Phipatanakul et al.) and is present in 100-fold higher concentrations in inner-city homes than in suburban homes.(Matsui et al.;Cohn et al.) *Mus mus* is a known cause of occupational asthma,(Schumacher, Tait, and Holmes;Gautrin et al.) and as many as 25% of inner-city homes have levels of airborne mouse allergen that are as high as levels seen in some occupational settings.(Matsui et al.) Furthermore, as many as 25-50% of inner-city children with asthma have evidence of IgE sensitization to mouse,(Matsui et al.) making them susceptible to developing symptoms upon exposure to mouse allergen.

All of this evidence points directly to household mouse allergen as an important cause of asthma symptoms and morbidity. These findings suggest that asthma symptoms and morbidity can be reduced among mouse-sensitized asthmatics if household mouse allergen levels can be reduced. In fact, the feasibility of reducing household *Mus mus* levels has already been demonstrated,(Phipatanakul et al.) but the effect of such an intervention on asthma outcomes is unclear. Thus, the evidence to date provides a strong rationale for the conduct of a randomized controlled trial (RCT) to determine the efficacy of a mouse allergen-targeted environmental intervention to reduce asthma morbidity.

#### 2.1.1 Description of the Intervention

The Integrated Pest Management (IPM) Group will receive an IPM strategy (extermination with rodenticide, traps, and sealing of holes and cracks), air filters, cleaning, mattress and pillow encasements, and education regarding pest control measures.(Phipatanakul et al.) The intervention procedures will be those that were used in the Boston pilot intervention study.<sup>(20)</sup> Rodenticide

tracking powder will be dusted in wall voids and pipe chases, which serve as rodent runways, and then sealed inside the walls with caulking material and copper mesh. Snap traps will be set away from children and pets in the kitchen, concentrating on areas around the refrigerator, trash, and stove. Additional traps will be set in the living room and bedroom. At the first IPM visit, targeted cleaning will be performed focusing on the kitchen and the bedroom to remove allergen reservoirs. In addition, room air filters will be placed in the bedroom and TV/living room. Allergen-proof mattress and pillow encasements will be placed on the child's mattress and pillow by the research assistant since the bed is a known reservoir for mouse allergen.(Chew et al.) The setting of traps and application of rodenticide and sealing of cracks and holes will be performed in a standardized manner by professional exterminators who will be trained in conducting interventions for research purposes. Research study staff will be responsible for placement of mattress and pillow encasements, cleaning, deployment of room air filters, and education. After randomization, participants randomized to the IPM arm will receive 2 IPM visits, approximately 4 weeks apart. Thereafter, the IPM group will receive the IPM booster module, consisting of 2 IPM visits approximately 4 weeks apart, if there is evidence of mouse infestation observed by the research assistant or reported by the parent/guardian at the 3, 6, and 9 month home assessment visits.

### 2.1.2 Summary of Relevant Clinical Studies

In a Boston-based study, homes of 18 mouse-sensitized and exposed asthmatics were randomized to either the control or the IPM groups.(Phipatanakul et al.) There were 6 control homes, and 12 intervention homes that received IPM. The study was conducted over a 5 month time period, and settled dust *Mus m 1* levels dropped throughout the study period in the intervention group, but increased in the control group after an initial drop. Overall, *Mus m 1* levels dropped by more than 75% in the intervention homes, from a median level of 24.1µg/g to 2.8µg/g.

Although there was a greater decrease in both days of wheezing and short-acting beta agonist use in the intervention group as compared to the control group, the difference was not statistically significant. However, the study was primarily designed to assess the efficacy of IPM in reducing household *Mus m 1* levels, not to detect improvements in asthma-related outcomes. Ultimately, the study was underpowered for health outcomes analyses, having only 12 homes in the intervention group and 6 in the control group. However, the suggestion that symptom outcomes improved in the intervention group relative to the control group supports the notion that a mouse allergen abatement intervention may result in improved asthma outcomes.

### 2.1.3 Summary of Epidemiological Data

- ***Residents of Baltimore and Boston have among the highest prevalence rates of allergic sensitization to mouse and household *Mus m 1* exposure.***

**Table 1.** Mouse Sensitization and Household Mouse Allergen Levels in Baltimore, and Boston

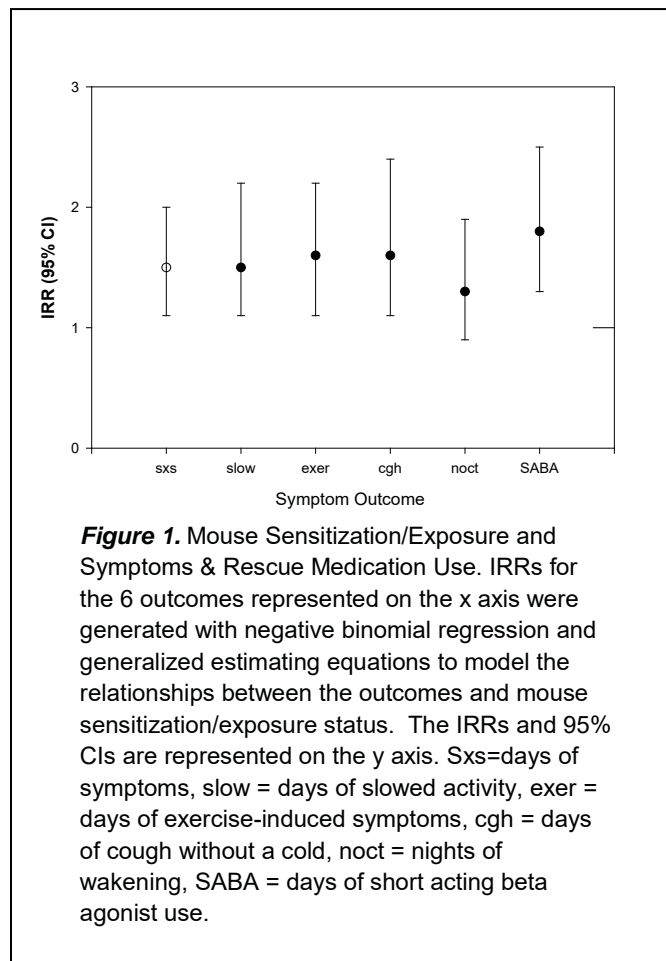
	IgE sensitization to mouse (%)	Settled Dust Mouse Allergen (µg/g) median (IQR)		
		Kitchen	Bedroom	% Detectable
NCICAS(Phipatanakul et al.;Phipatanakul et al.)	18	1.6 (BD*-618)	0.52 (BD-294)	95
Baltimore(Matsui EC et al.)	26-50	7.4 (0.9-31.6)	2.5 (0.6-8.8)	100
Boston	33-51	10.8 (0.3-43.6)	1.3 (0.3-4.8)	93

\*BD = below detection

IgE-sensitization to mouse is most common in Northeastern US cities, and Baltimore and Boston have among the highest sensitization rates. (Table 1) In addition, as many as 90-100% of these children are exposed to *Mus m 1* since *Mus m 1* is detectable in almost all inner-city homes.(Phipatanakul et al.;Phipatanakul et al.;Chew et al.;Matsui et al.) Because exposure is ubiquitous, almost all mouse-sensitized asthmatics residing in these cities are at risk of having mouse allergen-induced asthma symptoms. Furthermore, *Mus m 1* levels in these cities are 3-5 times higher than levels reported in the NCICAS, indicating that children in these cities are exposed to the highest levels in the US. If an effective means of reducing mouse allergen exposure can be identified, then a substantial proportion of asthmatic children could benefit, resulting in a decrease in symptom burden, medication use, and asthma-related health care use.

- ***Mouse allergen exposure is associated with asthma morbidity.***

In a prospective study of 127 preschool children with asthma in Baltimore City, there was a strong and consistent relationship between mouse allergen exposure and asthma-related outcomes, including both symptoms and asthma-related health care use.(Matsui EC et al.)



Children in the sensitized/higher exposure group experienced more symptoms than children in the other groups consistently across all symptom measures (Figure 1). In addition, the sensitized/higher exposure group had greater odds of asthma-related unscheduled doctor visit, emergency department visit, and hospitalization than children in the other groups. These findings persisted even after adjustment for multiple covariates, including cockroach allergen exposure and sensitization.

## 2.2 Rationale

### *US Urban Populations and Asthma Morbidity*

Urban neighborhoods are predominantly populated by socioeconomically disadvantaged populations, often of minority racial/ethnic backgrounds, and these underserved populations are more likely to have asthma, and suffer greater asthma morbidity than well educated, more affluent populations residing in suburban areas



or even urban areas of the same city (e.g. the asthma prevalence varies by 2-3 times between neighborhoods in NYC). For example, African Americans (AAs) are more likely to reside in the inner-city, and asthma is more prevalent and more severe in AAs. Although asthma prevalence rates and morbidity increased across all strata of the United States population from 1980-1999, the minority population continues to be affected to a greater extent than other racial/ethnic groups.(Mannino et al.)

Populations of Latino ethnicity also have a high prevalence of asthma and disproportionate morbidity, particularly in the Northeastern US.(Homa, Mannino, and Lara) In Boston, for example, hospitalization rates in AAs and Hispanics are 3-4 times higher than rates in non-Hispanic whites. Asthma-related mortality rates in Hispanic residents of New York City are 3 times higher than mortality rates among non-Hispanic whites.(Strunk, Ford, and Taggart)

More worrisome is the fact that we have made little progress in reducing these disparities because these differences in measures of asthma prevalence and morbidity have remained largely unchanged over the past decade. The importance of this public health problem is further underscored by the fact that the NIH has funded three multi-million dollar initiatives focused on asthma in this high risk population over the last 15 years.(Mitchell et al.;Morgan et al.;Liu)

### *Indoor Environmental Exposures in Urban Homes*

Pest allergens are common and found in high concentrations in the indoor environments in the inner-city. For example, Bla g 1 is detectable in 85% of inner-city homes, compared to 30% of suburban homes.(Eggleston et al.;Matsui et al.) Bla g 1 levels are also much higher in the inner-city, where 50% of homes have levels > 8U/g, compared to 12% of homes in the suburbs. It is not surprising, then, that cockroach allergen exposure has been linked to inner-city asthma morbidity,(Rosenstreich et al.) and, in fact, several groups are now actively investigating cockroach allergen abatement as a therapeutic intervention for asthma in this population.(Arbes, Jr. et al.;Eggleston et al.) Even though cockroach allergen exposure has emerged as an important factor in asthma morbidity and deserves the attention that it is receiving, it is clear that there are other major contributors to the ongoing asthma epidemic in urban centers, such as mouse allergen exposure.

### *Mouse Allergen and Asthma*

Mouse allergen (Mus m 1) has received much less attention than cockroach allergen, despite the fact that findings to date suggest that exposure to this pest allergen also plays an important role in asthma morbidity. Like Bla g 1, Mus m 1 is detectable in a very high proportion of inner-city homes and is present in 100-fold higher concentrations in inner-city homes than suburban homes.(Matsui et al.;Matsui et

al.;Matsui et al.) Mouse allergen exposure is also a well-recognized risk factor for occupational asthma. Approximately 30-50% of laboratory mouse workers have mouse-related allergic symptoms,(Schumacher, Tait, and Holmes) and 20% have skin test sensitivity to mouse.(Matsui et al.;Cullinan et al.;Aoyama et al.) As many as 30% of the skin test positive workers have occupational asthma.(Schumacher, Tait, and Holmes;Gautrin et al.) More compelling is the fact that Mus m 1 levels in some inner-city homes are comparable to levels found in occupational settings, where mouse allergen is known to cause both acute and chronic asthma.(Matsui et al.) In addition, the prevalence rate of mouse skin test sensitivity in the inner-city is similar to that found in occupational settings. In fact, cross-sectional studies of inner-city asthmatics have found mouse skin test sensitization rates of 17-50%.(Matsui et al.;Phipatanakul et al.) The fact that both the prevalence of mouse sensitization and the intensity of Mus m 1 exposure in the inner-city are similar to that observed in occupational settings where mouse allergen is a known cause of asthma symptoms provides a compelling rationale for a causal link between mouse allergen exposure and asthma in the inner-city.

Several studies to date have examined the relationship between household Mus m 1 exposure and asthma morbidity in children.(Phipatanakul et al.;Matsui EC et al.;Phipatanakul et al.) In the National Cooperative Inner-city Asthma Study (NCICAS), children who had a positive skin test to mouse and had  $>1.6\mu\text{g/g}$  of Mus m 1 in kitchen settled dust tended to report more days of wheezing and more nights of lost sleep than the others.(Phipatanakul et al.) In a Baltimore study, children who were sensitized to mouse and exposed to  $>0.5\mu\text{g/g}$  in bedroom settled dust had more days of symptoms and rescue medication use, and were at greater risk of asthma-related health care use than children who either were not sensitized or were exposed to lower levels,(Matsui EC et al.) and a recent multi-center study also found an association between mouse allergen exposure and asthma morbidity among mouse-sensitized children with asthma.(Pongracic et al.) Thus, there is growing evidence that household Mus m 1 exposure is an important contributor to asthma symptoms and morbidity that are observed in inner-city populations.

Ultimately, if we can demonstrate that reduction of exposure leads to improved asthma outcomes, then these findings would provide the strongest evidence to date of a causal relationship between Mus m 1 exposure and asthma morbidity. A randomized, controlled trial of an environmental intervention would be the optimal experiment to demonstrate causality, and such a clinical trial would directly test a public health intervention, potentially establishing an efficacious public health strategy for addressing the problem of household mouse allergen exposure.

The feasibility of reducing household mouse allergen levels has also now been established. This pilot work has been completed, and reducing household Mus m 1 levels by as much as 75% is feasible. In a Boston-based study, a 75-80% reduction

in settled dust *Mus m 1* levels was achieved in homes receiving IPM as compared to an increase in levels in control homes.(Phipatanakul et al.) This finding suggests that reduction of household mouse allergen exposure is a feasible public health approach to inner-city asthma, and that the next study should evaluate the efficacy of mouse allergen abatement in reducing asthma morbidity.

#### *Mus m 1 Abatement Strategies: Rationale*

As has been demonstrated for cat and cockroach allergens, removal of the allergen source is the most critical step in allergen abatement.(Wood et al.;Wood et al.;Wood et al.) For example, one must remove the cat(s) from the home in order to achieve a meaningful reduction in household *Fel d 1* levels. Similarly, for successful cockroach allergen abatement, the cockroaches must be exterminated. The same principle applies to successful mouse allergen abatement which is contingent upon extermination of the mice that are infesting the home.(Phipatanakul et al.)

A second objective of allergen abatement is to take measures to prevent re-entry of the source. Because mice can enter a home through an opening no bigger than the diameter of a pencil, sealing of any holes or cracks will prevent re-entry after extermination. In addition, meticulous disposal of food remains can substantially reduce food sources for rodents, so that they are less likely to seek refuge in the home. The combination of extermination, sealing of entry points, and meticulous housekeeping practices is referred to as integrated pest management (IPM), and is a standard approach to rodent extermination.(Phipatanakul et al.)

However, substantial reductions in animal allergen levels may not be observed for several months after the removal of the allergen source.(Wood et al.) This relatively slow decrease of allergen levels is likely due to the fact that the animal allergen has accumulated in reservoirs, and multiple rounds of cleaning and/or time are required for reservoir allergen levels to dissipate. Therefore, procedures aimed at reducing the allergen itself, such as extensive cleaning or air filters, may increase the rate of decline in allergen levels.

Because *Mus m 1* readily becomes airborne, room air filters that target mouse allergen-carrying airborne particles are a logical adjunct to IPM in a mouse allergen abatement intervention. Room air filters are commercially available, and are compact and easy to use – requiring no more from the user than to plug the device in, and change the filter approximately every 3-6 months.(Wood et al.;Eggleston PA et al.;Morgan et al.) These devices remove particles as small as 0.3 microns from the air, so that they are ideally suited for targeting *Mus m 1*, of which the bulk is found on particles less than 10 microns in diameter.(Ohman, Jr. et al.) In fact, room air filters have been shown to reduce airborne *Fel d 1* and *Can f 1*,(Green et al.;Wood et al.) allergens that have similar particle characteristics to *Mus m 1*. It is also intriguing

that the use of air filters in the Inner-city Asthma Study was associated with significantly improved asthma outcomes.(Morgan et al.) Even though airborne mouse allergen levels were not measured in the Inner-city Asthma Study (ICAS), sensitization to mouse in this population was relatively high (28%),(Gruchalla et al.) suggesting that the observed improvements in asthma may have been mediated by reductions in airborne Mus m 1 levels.

## 2.3 Potential Risks and Benefits

### 2.3.1 Potential Risks

- Questionnaires
  - The risk of the questionnaire is breach of confidentiality.
- Blood draw
  - The risk of blood drawing is discomfort, bruising, lightheadedness, and rarely infection.
- Lung function and exhaled nitric oxide testing
  - The risk of lung function and eNO testing is the discomfort of exhaling forcefully.
- Saliva Collection
  - No known risk associated with chewing parafilm.
- Buccal cell sample collection
  - There is no known risk associated with the collection of buccal cells using the cytology brushes
- Home visits
  - The risk of the home visit for collection of environmental data is breach of confidentiality.
- Skin testing
  - The risk of skin testing is primarily the discomfort from any positive skin tests, which would result in wheal and flare responses and pruritus. Approximately 2-3 in 10,000 skin prick tests results in allergic symptoms away from the site of the skin testing, such as sneezing, rhinorrhea, or rash.(Lin et al.;Valyasevi, Maddox, and Li) Very rarely, an individual who experiences this type of reaction may develop life-threatening symptoms such as persistent coughing or wheezing. A study published in 1987 reported six fatalities from skin testing since 1945.(Lockey et al.) From 1985-1989 there were no fatalities from skin testing reported,(Reid et al.) and one fatality was reported from 1990-2001.(Bernstein et al.)
- Rodenticides

- Rodenticides are long-acting anticoagulants and there is a risk of developing a coagulopathy with intentional ingestion. There have also been case reports of accidental ingestion of these products resulting in coagulopathy.(Smolinske et al.;Watts, Castleberry, and Sadowski) Several prospective studies of accidental rodenticide ingestion in children indicate that almost all accidental ingestions can be managed by observation at home since they rarely result in coagulopathy. In one series, among more than 200 children with reported accidental ingestion of superwarfarin, two patients had an INR of 1.5 or greater, both of whom were asymptomatic.(Ingels et al.) In another series, the National Poisons Unit for London reported no serious adverse outcomes over a five year period, during which they received an average of 200 reports per year.(Kanabar and Volans) Of 542 reports of accidental ingestion by children to a US poison control center, follow-up coagulation laboratory tests did not detect any significant coagulation abnormalities, no child developed bleeding complications, and no child required or received vitamin K.(Mullins, Brands, and Daya) For the purposes of this study, rodenticides will be used by licensed exterminators who will be trained specifically on procedures for the MAAIT. Rodenticide will only be placed in areas that are not accessible to children or pets. Specifically, rodenticide will be placed in holes and cracks, which will then be sealed to prevent extrusion of the rodenticide into the home or access to the rodenticide by children or pets.
- Cleaning agents and caulking agents
  - Commercially available, non-bleach cleaning agents (e.g. 409®, Fantastik®) will be used during the cleaning that occurs during the first IPM visit. Commercially available foam caulking agents (e.g. Pur-fill®, Hilti) will be used to seal holes and cracks during the IPM visits. Both of these agents contain potential irritants that could cause asthma symptoms. The risk of developing asthma symptoms serious enough to require emergency medical attention is very small. The cleaning agents will be used as directed by the manufacturers. The caulking agents will be used as directed by manufacturer instructions.

### 2.3.2 Potential Benefits

The potential benefits for the study participant includes receiving an assessment of allergic sensitivities, assessment of home environmental exposures, room air filters, extermination and cleaning, as well as education regarding environmental control practices. The study participants may also

benefit from improved asthma control. The potential benefits to others include the identification of a new cause (or causes) of asthma symptoms and morbidity in the inner-city, and identification of a method to reduce asthma morbidity in mouse-sensitized asthmatics with household mouse allergen exposure. This knowledge will impact the way physicians provide care for inner-city asthma patients, and will impact policy decisions regarding environmental interventions to reduce household exposures that may trigger asthma symptoms. This information will directly impact the medical care provided to this patient population, and could corroborate the findings of epidemiologic studies, confirming the role of a prevalent indoor exposure in the ongoing inner-city asthma epidemic.

### 3. STUDY OBJECTIVES

#### 3.1 Primary Objective

To determine the efficacy of this mouse allergen-targeted environmental intervention among mouse-sensitized children with asthma to improve asthma outcomes.

#### 3.2 Secondary Objectives Overview

The study is not powered for hypothesis testing on secondary endpoints. Instead, all secondary endpoints will be treated as supportive and findings should be interpreted with caution.

##### 3.2.1 Secondary objectives:

To determine the efficacy of this mouse allergen-targeted environmental intervention among mouse-sensitized children with asthma to:

- (a) Reduce additional direct measures of mouse allergen exposure including bed, bedroom floor, and kitchen settled dust mouse allergen levels and airborne mouse allergen levels.
- (b) Reduce serum levels of mouse allergen-specific IgG, a biomarker of Mus m 1 exposure, and serum levels of mouse allergen-specific IgE, a potential biomarker of Mus m 1 exposure.
- (c) Improve additional clinical outcomes, including additional symptom outcomes, rescue medication use, health care use, and asthma control.
- (d) Improve physiologic and inflammatory markers of asthma disease activity, including:
  - (1) FEV1
  - (2) FEV1/FVC
  - (3) eNO
  - (4) reversible airways obstruction, defined as % change in FEV1 following administration of short-acting bronchodilator.

## 4. STUDY DESIGN

### 4.1 Description of the Study Design

MAAIT is a randomized, controlled, parallel group clinical trial designed to assess the efficacy of a mouse-targeted home environmental intervention in reducing home mouse allergen levels and improving asthma control in mouse-sensitized children with asthma. MAAIT is a multi-center environmental intervention study with two groups: (1) IPM Group and (2) Education Group. Children 5-17 years of age with moderate to severe asthma who are sensitized to mouse and have high home mouse allergen levels will be enrolled. Three hundred-fifty children will be randomized, as many as 125 from the Boston site and as many as 250 from the Baltimore site, and enrollment will be completed at 36 months. Each randomized participant will be in the study for a total of about 13.5 months, approximately 6 weeks prior to randomization and 12 months following randomization.

Potential study participants will be screened over the telephone and those who meet study criteria for uncontrolled asthma will be scheduled for a baseline/screening study visit in the clinic. At the screening visit, after participants consent and assent to participate, verification that the subject meets inclusion and exclusion criteria will be done. At the screening visits, all participants who meet the inclusion and exclusion criteria, as determined by the verification form will provide a urine sample. Female participants with a history of menstruating will have a urine pregnancy test performed. A positive pregnancy test will exclude them from continuing in the study. Skin prick testing and specific IgE testing will not be performed before pregnancy test results are known.

At the screening visit, participants  $\geq 12$  years old will have their urine screened for cotinine as an assessment of smoking status. A positive urine screen for active smoking will not exclude the participant from continuing with CV1, but will exclude them from continuing with study activities beyond CV1. All CV1 data, except buccal swabs, will be collected in this population for ancillary studies being performed related to saliva collection. The ancillary studies are related to the role of salivary inflammatory profiling in phenotyping asthma (see salivary collection section of protocol).

All other participants who have had inclusion/exclusion confirmed by the verification form, are not pregnant and not active smokers will complete all clinic visit procedures which include: skin prick testing, venipuncture for determination of mouse allergen-specific IgE and IgG levels, pre- and post-bronchodilator spirometry, exhaled nitric oxide (eNO), saliva collection, buccal swabs and completion of questionnaires. Participants who are pregnant, active smokers (as determined by NicAlert™), and/or



not mouse sensitized (negative skin prick test to mouse and no detectable mouse-specific IgE) will not be eligible to continue with the study. (See MOP).

Participants who meet the clinical eligibility criteria will be scheduled for a home visit within approximately 3 weeks following the screening clinic visit to determine mouse allergen exposure status. At the home visit, a home assessment will be conducted, and settled dust and air samples will be collected from the participant's bedroom. The air samples will be collected to have a baseline determination of airborne dust. The bed settled dust sample will be analyzed for Mus m 1 content by ELISA to determine if the participant meets the exposure eligibility criterion ( $\geq 0.4\mu\text{g/g}$  Mus m 1 in bed dust or  $\geq 0.5\mu\text{g/g}$  Mus m 1 in bedroom floor dust). Participants meeting both clinical and exposure eligibility criteria will be randomized within approximately 3 weeks of the home visit to the IPM Group or the Education Group.

The IPM Group procedures will include:

- Targeted cleaning to remove allergen reservoirs
- Extermination (placement of traps and application of rodenticide)
- Pest management education (education regarding setting traps, sealing holes and cracks, and housekeeping practices (see MOP))
- IPM Education
- Sealing of holes and cracks in the home
- Two room air filters will be placed in the participant's bedroom and TV/living room.
- Allergen-proof mattress and pillow encasements

The Education Group procedures will include:

- Pest management education (education regarding setting traps, sealing holes and cracks, and housekeeping practices (see MOP)).  
Upon completion of the study: Extermination, sealing of holes and cracks, allergen-proof mattress and pillow encasements, cleaning, and room air filters will be offered to this study group.

The overall study scheme is depicted in Figure 2. The IPM Group will receive two intervention visits at approximately 2 and 6 weeks. The first IPM visit will also include cleaning that will focus on removing allergen reservoirs. Subsequent IPM visits will occur if there is evidence of mouse infestation or parent/caregiver report of infestation at home assessment visits at 3, 6, and 9 months. The Education Group will receive one home visit during which they will receive Pest management education at  $2\pm 3$  weeks after randomization. At completion of the 12 month visit, the Education Group will be offered one IPM visit, mattress and pillow encasements, cleaning, and 2 room air filters..

- 6 wks	Screening Clinic
-3 wks	Baseline Home (HV1)
0	Randomization

**Figure 2. MAAIT Study Scheme**

		HOME	CLINIC	TELEPHONE	IPM GROUP	EDUCATION GROUP
2wks ±3 wks					IPM Visit + Cleaning + Education	Pest Management Education Visit
6wks ±3wk					IPM Visit	
3mos ±3wks		X*		X		
6mos ±3wks		X*	X			
9mos ±3wks		X*		X		
12mos ±3wks		X	X			
* if evidence of active infestation on home assessment in homes randomized to IPM GROUP, triggers IPM module protocol <b>IPM MODULE consists of:</b> (1) IPM Visit <b>and</b> (2) IPM BOOSTER Visit approximately 4 weeks later						

Participants will be followed for approximately 12 months. Home visits will be conducted every 3 months to assess settled dust *Mus m 1* levels and to inspect the home for evidence of infestation. Buccal Swabs, for DNA only, will be collected during the 3 and 9 month home visits if the study participant is at home. At 6 and 12 months, there will be clinic visits to collect clinical outcome data. The clinical outcome data collected at the clinic visits will include symptoms, rescue medication use, health care use, prednisone bursts, eNO, and pre- and post-bronchodilator spirometry. In addition, venipuncture will be performed for determination of mouse allergen-specific IgE and IgG levels at the screening and 12 month visits and buccal swabs for DNA and RNA samples will be collected to assess epigenetic changes associated with the study intervention. Saliva will be collected for measurement of inflammatory markers to determine if the intervention is associated with changes in salivary inflammatory markers. Telephone visits will be conducted at 3 and 9 months to collect symptom, medication, and health care use data. At the screening visit,

blood will also be drawn for a complete blood count with differential (CBC) and a C-reactive protein level for a cross-sectional ancillary study examining relationships between obesity and inflammatory markers among asthmatics. At the screening visit and CV3, at the Baltimore site only, blood may be drawn for an ancillary study examining the relationships between weight, gender, and immunologic characteristics of peripheral blood mononuclear cells in participants age 12-17. The primary outcome is maximum days of asthma symptoms over a two week period. Secondary outcome measures for mouse allergen exposure include settled dust bed, bedroom floor and kitchen settled dust allergen levels, airborne mouse allergen levels and mouse allergen-specific IgG and IgE levels. Clinical secondary outcome measures include rescue medication use, exacerbations (defined as prednisone bursts), asthma-related health care utilization, and additional symptom outcomes (nocturnal symptoms, exercise-related symptoms, and cough without a cold). Additional secondary physiologic and inflammatory outcome measures will include: pre- and post-bronchodilator lung function, exhaled nitric oxide. Additional analyses will also be performed to examine clinical outcomes as a function of change in mouse allergen levels to determine if improved clinical outcomes are linked to decreases in home mouse allergen exposure.

Mouse allergen levels will be measured by ELISA in the Environmental and Biologic Sample Core (EBSC) at Columbia University under the direction of Dr. Matt Perzanowski. Mouse-specific IgE levels will be measured using the ImmunoCAP system in the EBSC. Mouse-specific IgG levels will be measured in a central laboratory at the Sanquin Laboratory in Amsterdam, The Netherlands, under the direction of Dr. Rob Aalberse. Lung function and eNO will be assessed using the same brand and model equipment across all sites. Clinical outcome data will be collected by questionnaire. Study staff will be trained on procedures and SOPs developed to ensure that the same methodology is used across sites.

Home Mus m 1 levels and serum mouse-specific IgG and IgE levels will be compared between the IPM and Education groups to determine if the intervention reduced mouse allergen exposure. In addition, asthma outcome data will be compared between the IPM and Education groups to determine if the intervention resulted in reduced asthma symptoms and medication use, reduced asthma-related health care use, reduced exacerbations, improved pulmonary function, or reduced eNO levels. Clinical outcomes will also be analyzed as a function of change in the settled dust and airborne mouse allergen levels to determine if clinical improvement is associated with decreases in mouse allergen exposure.

## 4.2 Study Endpoints

### 4.2.1 Primary Endpoint

The primary endpoint is days of asthma symptoms.

- Maximum symptom days over two weeks will be compared between IPM and Education Groups.

### 4.2.2 Secondary Endpoints

Secondary endpoints include:

- Additional direct measures of mouse allergen exposure:
  - Airborne mouse allergen levels
  - Bed settled dust mouse allergen levels
  - Bedroom floor settled dust mouse allergen levels
  - Kitchen settled dust mouse allergen levels
- Biomarkers of mouse allergen exposure:
  - Mouse-specific IgG levels in serum
  - Mouse-specific IgE levels in serum.
- Other measures of asthma symptoms,
  - Days of rescue medication use/2 weeks;
  - Days of cough without a cold/2 weeks;
  - Days of exercise-induced asthma symptoms/2 weeks;
  - Nocturnal symptoms/2 weeks.
  - Days of slowed activity/2 weeks.
  - Asthma control, assessed by the Asthma Control Test
- Pulmonary function:
  - FEV1,
  - FEV1/FVC
  - percent change in FEV1 after short-acting bronchodilator.
- Pulmonary inflammation, assessed by exhaled nitric oxide.

### 4.2.3 Additional Endpoints

The following research questions will also be addressed::

- (1) In the IPM group, is the change in mouse-specific IgG and/or IgE levels correlated with:
  - The change in direct measures of mouse allergen exposure?
  - Change in clinical outcomes?
- (2) In the IPM Group, does the degree of exposure reduction modify the effect of the intervention on asthma outcomes?

The degree of exposure reduction will be defined using cut points previously suggested as thresholds for successful allergen reduction and are as follows:

- $\geq 90\%$  reduction in mouse allergen in bed or bedroom floor dust samples
- $\geq 90\%$  reduction in mouse allergen in airborne samples
- $\geq 75\%$  reduction in mouse allergen in bed or bedroom floor dust samples
- $\geq 75\%$  reduction in mouse allergen in airborne samples
- $\geq 50\%$  reduction in mouse allergen in bed or bedroom floor dust samples
- $\geq 50\%$  reduction in mouse allergen in airborne samples

(3) In the IPM group, does the presence of other indoor allergen sensitivities and exposures modify the effect of the intervention on asthma outcomes?

The presence of other indoor allergen sensitivities and exposures will be defined using common indoor allergen sensitivities and previously suggested cut points for clinically relevant exposure thresholds as follows:

- +SPT or specific IgE to cockroach AND cockroach allergen levels  $\geq 2$  U/g in bed or bedroom floor dust samples
- +SPT or specific IgE to cat or dog AND cat or dog allergen levels  $\geq 2$  U/g in bed or bedroom floor dust samples
- +SPT or specific IgE to dust mite AND dust mite allergen levels  $\geq 2$  U/g in bed or bedroom floor dust samples
- Any of the above sensitization and exposure criteria
- Other cut points will also be determined *a priori* and interactions will also be tested using exposure as a continuous variable.

(4) Does the degree of sensitization to mouse modify the effect of the intervention?

Study participants will be stratified by mouse-specific IgE as follows: (1)  $<5.0$ ; (2)  $5.0-20.0$ ; (3)  $>20.0$  kU/L, and efficacy of the intervention within each of these strata will be examined.

The IPM group will be stratified by these three potential effect modifiers ((1) degree of exposure reduction; (2) presence of other indoor allergen sensitivities and exposure; and (3) degree of mouse sensitization) and the effect of each of these on the impact of the intervention on the primary and secondary outcomes listed above will be examined.

In addition, salivary inflammatory markers will also be measured to:  
(1) assess the utility of salivary inflammatory profiling to identify novel asthma phenotypes

(2) examine the impact of the mouse allergen targeted intervention on salivary inflammatory markers (should the inflammatory profile change over time).

Buccal DNA and RNA samples will be assessed to determine if epigenetic changes of inflammatory and immune-related genes are associated with the study intervention.

At the screening visit, blood will also be drawn for a complete blood count with differential (CBC), a C-reactive protein level for a cross-sectional ancillary study examining relationships between obesity and inflammatory markers among asthmatics.

Urinary and serum oxidative stress and inflammatory markers, including urinary isoprostane and serum cytokines, will also be measured to examine relationships between obesity and these inflammatory markers.

At the Baltimore site only, peripheral blood mononuclear cells may be obtained from participants 12 years and older at CV1 and CV3. Obesity, in some studies, is a risk factor for more severe asthma, and one working hypothesis for this observation is that adipocytes serve to promote differentiation of monocytes to alternatively activated macrophages (AAMs), which promote scarring and Th2 inflammation. The development of AAMs is favored by alternations in IL-4/IL-13 receptor signaling in monocytes which may be mediated by molecules produced by adipocytes. This potential link between obesity and Th2 inflammation may, at least in part, explain associations between obesity and asthma.

Therefore, the aim of this ancillary project is to examine IL-4/IL-13 signaling responses, their negative regulation and changes in gene expression in PBMCs from children with allergic disease. We will use Western blotting and intracellular phospho-flow cytometric analysis to examine signaling pathways and quantitative RealTime PCR to evaluate changes in gene expression to determine whether PBMCs from children with asthma show alterations in receptor expression and how these changes alter downstream signaling events and gene expression initiated by IL-4 and IL-13. Our collaborator, Dr. Heller, has already examined these PBMC characteristics from healthy controls, so that data from healthy controls will be compared to data from our asthmatic population. More importantly, we will also examine how obesity affects the above parameters and the differentiation of peripheral blood monocytes to macrophages. The specific questions to be answered are:

1. Does obesity alter expression of IL-4 receptor chains on circulating PBMCs (monocytes, T-cells) in adolescents with asthma? Expression of IL-4 receptor chains on PBMCs will be compared between obese and non-obese adolescents with asthma.
2. Do the signaling pathways in PBMCs downstream of the IL-4R (e.g. STAT6, IRS-2) show either enhanced activation to IL-4/IL-13 or

insensitivity to the negative regulatory mechanisms in obese adolescents with asthma (vs. non-obese adolescents with asthma)?

3. Do PBMCs from obese adolescents with asthma have a greater ability to spontaneously develop into alternatively-activated macrophages or classically-activated macrophages? Or express elevated markers of alternative activation (MMR, YKL-40)? These outcomes will be compared between obese and non-obese asthmatics.
4. Are IL-4/IL-13 induced genes expressed in monocytes derived from obese adolescents with asthma more resistant to steroid suppression than non-obese adolescents with asthma?
5. Are monocytes from obese females more likely to spontaneously differentiate into AAM than those from non-obese females and/or obese males?

Ad hoc analyses may be conducted to explore the impact of the intervention on Th2 inflammation parameters in obesity and non-obese study population.

## 5. STUDY POPULATION

### 5.1 Description of the Study Population

#### 5.1.1 Participant Inclusion Criteria

- Males and females who are 5-17 years of age, inclusive, at the screening visit
- Have physician-diagnosed asthma at least 1 year prior to the screening visit, or asthma symptoms for at least 1 year
- Meet criteria for current persistent asthma defined as either:

(a) On a long-term controller medication for asthma, or

(b) Meet NAEPP guideline requirements for persistent disease:(NAEPP expert panel report. Managing asthma during pregnancy: recommendations for pharmacologic treatment-2004 update)

1. Asthma symptoms 3 or more days per week over the past 2 weeks OR
2. Nocturnal asthma symptoms at least 3 times in the past month

- Have evidence of uncontrolled disease as defined by at least one of the following:
  1. One asthma-related unscheduled visit to an emergency department (ED), clinic or urgent care facility in the previous 12 months
  2. One asthma-related overnight hospitalization in the previous 12 months
  3. One or more bursts of oral corticosteroids in the previous 12 months
- Sensitized to mouse
  1. either a positive SPT (net wheal  $\geq 3$ mm) or
  2. a positive mouse-specific IgE tested, as quantified using the ImmunoCAP mouse urine reagents ( $\geq 0.10$  kU/L)
- Have  $\geq 0.40$   $\mu\text{g/g}$  of Mus m 1 in the bed settled dust sample or  $\geq 0.50$   $\mu\text{g/g}$  of Mus m 1 in bedroom floor settled dust.
- Reside within a geographic area of the study site so that home visits are feasible. In Baltimore, participants must reside within the beltway, in Boston, participants must reside within the Greater Boston area.



- Have no plans to move within the upcoming 12 months

### 5.1.2 Participant Exclusion Criteria

- Pregnancy
- Lung disease, other than asthma, that requires daily medication
- Cardiovascular disease that requires daily medication, excluding hypertension
- Taking a beta-blocker
- Currently taking Xolair
- Currently receiving Immunotherapy (allergy shots)
- Sleeping in another home 4 or more nights/week
- Active smoker defined as a positive urine screen for high levels of urine cotinine ( $\geq 3$  on the NicAlert) or reported active smoker for subjects  $\geq 12$  years of age
- Unable to access areas of home necessary to conduct extermination
- Home in extensive state of disrepair as determined by Study Coordinator/PI judgment

## 5.2 Strategies for Recruitment and Retention

Study participants will be recruited using approaches that have been successful at each study site. There are primary recruitment approaches for each site that are expected to meet recruitment needs. However, enrollment will be reviewed weekly and on the Steering Committee conference call each month. Every six months, we will determine if enrollment is at least 80% of the target. If enrollment is not meeting this benchmark, then secondary recruitment resources will be employed. If, six months after employing secondary recruitment resources, enrollment is still  $<80\%$  of the target, then an additional site may be added. University of Maryland is an attractive additional site for several reasons: first, the patient population that it serves is highly mouse allergen exposed; second, it is on the west side of Baltimore City, so reaches a different geographic area than Hopkins; and third, resources and personnel at the Hopkins site could easily be leveraged for a site in the same city. A second alternative site is the NYC-Columbia University site which has been involved in the planning of the MAAIT since inception and will already be a laboratory core site.

**In Baltimore**, three primary recruitment approaches will be employed.

The *first* approach will be to recruit participants who have completed the Mouse Allergen and Asthma Cohort Study (MAACS), an R01-funded observational study of home mouse allergen exposure and asthma. Study participants are 5-17 years of age and have moderate to severe asthma at enrollment, so are likely to meet eligibility criteria for MAAIT. In addition, the last participant was enrolled in early summer 2009, so that the last participants are anticipated to complete the study in

summer of 2010, providing optimal timing for transition from the MAACS to MAAIT. **Moreover, >80 participants in MAACS meet asthma severity, sensitization and exposure eligibility criteria for MAAIT, representing almost half of the total participants that will be needed for MAAIT.** Second, participants will be recruited from the Future Subjects Database, a database of over 700 individuals who have previously participated in asthma-related studies. Because the Baltimore site has access to skin test records of these individuals, mouse-sensitized individuals will be targeted for recruitment. The third approach at the Baltimore site will be recruitment through the emergency department. The Pediatric Emergency Department has over 24,000 visits annually, and primarily serves patients from Baltimore City. Patients who have recently visited the Johns Hopkins ED for an asthma exacerbation will be identified and contacted to determine interest and potential eligibility for the study. In recruitment for previous studies, 10-20 potential study participants have been identified each week through this mechanism.

If the first three recruitment approaches are not sufficient to meet recruitment goals, the Baltimore team will recruit patients from Johns Hopkins Community Physicians (JHCP), a multi-clinic primary care health system that serves Maryland. JHCP provides internal medicine, family practice, pediatrics and OB/Gyn practitioners, as well as a wide range of other primary care services to over 400,000 patients annually. JHCP operates three clinics covering a catchment area that includes both Eastern and Northern Baltimore City. The Baltimore site has also had success recruiting from community hospital emergency departments such as St. Agnes and Bayview – both of which are affiliated with Hopkins.

**In Boston**, Dr. Phipatanakul will employ three primary recruitment approaches. First, study participants will be recruited from the Children's Hospital Asthma clinic which sees over 5000 patients annually with asthma and allergic diseases. There is an actively maintained database of the allergy skin testing results of these patients and hundreds of children who have participated in previous asthma and allergy-related studies. There are >250 children with asthma and documented mouse sensitization in this database, so that this database should be the primary recruitment source for the MAAIT. Second, Dr. Phipatanakul is the PI of an NIH/NIAID R01-funded study, asthma study entitled "Allergens in Inner-City Schools and Childhood Asthma" (AI-073964) or School Inner-City Asthma Study (SICAS). This study is recruiting 150 students per year over 4 years for a total of 600 children. Each group of 150 children will be followed for one year and will have data on allergen skin testing, home and school allergen exposure, lung function data, and asthma morbidity data. Eligible children who have completed follow up in SICAS will be a rich source of recruitment for the MAAIT in that **much of the eligibility criteria will have been determined from enrolling in SICAS.** Furthermore, Dr. Phipatanakul will have access to screening surveys distributed to thousands of children in entire schools which will aid in identifying asthmatic children who may be

interested and qualify for MAAIT who have not necessarily enrolled in SICAS. There are currently >1000 screening questionnaires in the database and 150 enrolled participants with the expectation that >1000 additional screening questionnaires and 150 new participants per year will be accrued. Third, study participants will be recruited from the South End Community Health Center, which is staffed by asthma physicians from Boston Children's Hospital. The South End Community Health Center serves primarily Latino and African-American patients of low-income in the Dorchester/ Roxbury inner-city area. This community health center provides care for 15,000 patients, 62% of whom are pediatric patients. Approximately 90% of the patients are low-income and 65% are Latino/Latina. Asthma physicians provide full asthma evaluations, including allergy skin testing. The ability to access skin test data, so that mouse sensitized patients can be targeted for recruitment, will greatly facilitate the recruitment process. In addition, study participants for previous studies, including the Boston site pilot mouse allergen intervention study, were successfully recruited from this site.

With an estimated sample size of 125 from the Boston site and 225 from the Baltimore site (350 total), we anticipate that recruitment and enrollment will be completed over the first 36 months of the study.

**Retention:** The rate of drop-outs will be minimized by the use of telephone reminders for upcoming appointments, collection of multiple types of contact information for the participant, conducting home visits to locate study participants who are at risk of dropping out, and providing incentives for completion of study visits. Transportation to and from clinic visits will also be provided. The incentive scheme is as follows: For completion of every clinic visit, the parent/guardian will receive 40 dollars, plus an additional 10 dollars for bringing the child's asthma medications to the study visit. For completion of each telephone questionnaire, the parent/guardian will receive 10 dollars. For completion of each home visit, the parent/guardian will receive 25 dollars. The child will receive a gift card or age-appropriate toy valued at 10-15 dollars after completing each clinic visit and a small age-appropriate toy/item, valued at 1-3 dollars, if he/she completes the 3 and 9 month home visit buccal swab.

In addition to the above incentives, the Baltimore and Boston sites will give the following bonus incentives that are specific to the needs in each site locations:

Baltimore: A 30 dollar bonus will be given if **all** visits through the 6 month time point are completed. The 6 month visits are Clinic Visit 2 and Home Visit 3. Another 30 dollar bonus will be given if **all** visits from the six month time point to the 12 month time point are completed. The 12 month visits are Clinic Visit 3 and Home Visit 5.

Boston: An additional 20 dollars will be given if participants come to Clinic Visits 1, 2, and 3 within 15 minutes of their scheduled start time and do not need to reschedule.

## 6. STUDY AGENT/INTERVENTIONS

### 6.1 Study Agent Acquisition

#### 6.1.1 Integrated Pest Management

##### 6.1.1.1 Preparation and Administration of Study Intervention

In order to ensure the provision of high quality IPM services according to the study protocol, IPM companies that serve Baltimore and Boston will partner with the study teams. Each company will be licensed and insured and will be screened for general overall quality of its work, reputation, and enthusiasm for the study. Each company identified as a partner will provide approximately 2-3 IPM technicians who will undergo training for the MAAIT IPM protocol along with the IPM Company President/CEO and/or operations manager, whichever is most appropriate.

**Overview:** Each IPM Module will include two home visits. The first visit of each module will be conducted by a Research Assistant (RA) and an IPM technician (IT) from the IPM company. The two IPM visits will be 4±3 weeks apart for the initial module that is delivered after randomization. Subsequent IPM modules will occur if there is evidence of infestation or report of infestation at the 3, 6, and 9 month home assessment visits, and will consist of two IPM visits that will be 4±3 weeks apart. There will be no cleaning included in subsequent IPM modules. The control group will receive one IPM intervention visit as described below after completion of the study.

**IPM Intervention Visit 1:** The IPM intervention team will include the Research Assistant (RA) and IPM Technician (IT). The RA and IT will encourage and guide the family's participation in the visit. The ***Pest Monitoring Report Form*** will be used to document a walk through the living quarters to sketch a layout of the home and document visual evidence of infestation and holes/cracks in the structure. IPM procedures will include placement of traps, application of rodenticide and sealing of holes and cracks as specified in the MOP.

**Focused Cleaning:**

At the first IPM visit, the research assistant will complete a focused cleaning aimed at (1) removing allergen reservoirs; and (2) removing clutter to aid the IPM technician. Procedures will include removal of dead mice from traps, removal of trash and clutter, removal of mouse

droppings, wet mopping of floors with hard surfaces and vacuuming carpeted floors with HEPA filtered vacuum cleaner.

**Education:**

The research assistant will deliver the same educational module to both IPM Group and Education Group participants. The Education SOP which can be found in the MOP details the information to be covered and the Education Form will serve as documentation of delivery of the Education Module. In addition, the research assistant will have examples of food storage containers, and traps on hand to show the parent/guardian.

The research assistant will review the approaches to reducing mouse allergen levels – source removal, prevention of re-entry, and cleaning of allergen reservoirs - and document that the topics were reviewed with the parent/guardian.

**IPM Intervention Visit 2 (IV 2):** 4±3 weeks after IV1, the Booster Visit will follow the same protocol as Intervention Visit 1. This visit may be done by the IPM technician only.

**6.1.1.2 Study Intervention Accountability Procedures**

Several procedures will be in place to ensure adherence to the protocol, including timely and appropriate delivery of IPM services. These procedures include:

1. IPM technicians will undergo training and certification prior to enrollment. The training and certification will be led by Mr. Don Rivard.
2. IPM technicians will be required to complete all study forms and document procedures; forms will be reviewed by the Study Coordinator and 10% will be reviewed by Don Rivard.
3. A Research Assistant or Study Coordinator from the study team will be present during each Visit 1 of each IPM module to facilitate the IPM visit.
4. Annual site visits will be conducted by Mr. Rivard to observe at least 1 IPM visit at each site.
5. IPM Company President or Operations Manager will participate in regular conference calls to ensure timely communication of any protocol deviations or issues that need to be addressed.

## **6.1.2 Portable Room Air Filters**

### **6.1.2.1 Formulation, Packaging, and Labeling**

Two 3M Filtrete® portable room air filters will be deployed in each home randomized to the IPM Group (appropriate for rooms up to 170 square feet). Units will be deployed in the child's bedroom and in the TV/living room. The units will be made available in commercial packaging that includes an instruction manual printed in English and Spanish. The control group will be offered air filters after completion of the study.

### **6.1.2.2 Study Agent Storage and Stability**

Air filter units will be stored at each site according to the manufacturer's recommendations. Air filter units will be checked for functionality prior to deploying in a home.

### **6.1.2.3 Study Product Accountability Procedures**

According to the manufacturer, the portable air purifier units have a particle collection efficiency of 99.9% for particles as small as 0.3 microns. All filters will be replaced at each home visit, every 3 months, as recommended by the manufacturer's instructions.

## **6.1.3 Mattress and Pillow Encasements**

Commercially available mattress and pillow encasements will be obtained from one manufacturer. The research assistant will install the mattress and pillow encasements on the child's mattress and pillow at the first IPM visit for the intervention group. Subjects randomized to the Education group will be offered mattress and pillow encasements after completion of the study.

## **6.2 Assessment of Participant Compliance with Study Intervention**

Compliance with each of the study intervention procedures will be assessed.

- For IPM visits, the visits and dates of the visits will be documented. In addition, the IPM Technician will document the number of traps placed and application of rodenticide. Third, post-IPM visit telephone calls will be made to parents/guardians to assess the number of mice trapped since the IPM visit.

- For the portable air filters, parents/guardians will be administered a questionnaire at each clinic visit and telephone visit to assess reported functionality of the units and average use of the units. A research assistant will assess the units at the home visits to document functionality and whether the unit is on at the time of the home visit. A random 10% sample of air filters will be monitored using electric current data loggers that record the presence of an electric current every hour.
- For mattress and pillow encasements, the research assistant will document the presence/absence of these by direct observation at home assessment visits.

### **6.3 Concomitant Medications and Procedures**

Medications and/or procedures prescribed by the participant's health care provider or initiated and obtained by the participant's parent/guardian are permitted.

### **6.4 Precautionary and Prohibited Medications and Procedures**

There are no precautionary or prohibited concomitant medications or procedures, but all will be documented at either each clinic and telephone visit (medications) or environmental procedures (via questionnaire to parent/guardian and home assessment visits). Antihistamines will be discontinued for 3-5 days prior to the screening clinic visit for skin testing.

### **6.5 Rescue Medications**

All asthma management will continue as indicated by the subject's physician. Rescue medications will not be provided by the study. Participants who do not have a health care provider will be referred to one.



## 7. STUDY PROCEDURES/EVALUATIONS

### 7.1 Clinical and Home Environmental Evaluations

Clinical and Environmental Evaluations will occur during three types of study events: clinic visits, telephone questionnaires, and home assessment visits. Clinic Visits will occur at screening, 6 and 12 months. Telephone questionnaires will be administered at 3 and 9 months to capture interval clinical, medication, and environmental history. Home assessment visits will occur at screening, 3, 6, 9, and 12 months.

#### 7.1.1 Screening Clinic Visit (CV1)

Skin testing: Allergy skin testing will be performed to 14 allergens, including mouse epithelial extract, using the MultiTest II device (Lincoln Diagnostics, Decatur, IL). The allergen extracts to be used are: dog, cat, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, rat epithelia, German cockroach, American cockroach, mouse epithelia, tree mix, grass mix, *Alternaria*, *Aspergillus*, common ragweed, and *Cladosporium* (Greer Laboratories, Lenoir, NC). Skin tests will be performed according to procedures detailed in the Manual of Procedures. Subjects who return to the study for rescreening will not have to undergo skin testing a second time. Skin test results performed by the study team within the last year may be used for inclusion criteria.

eNO: eNO is a known marker of pulmonary inflammation and will provide a non-invasive means of assessing pulmonary inflammation in a large cohort that includes children.(Buchvald et al.;Cardinale et al.;Pijnenburg et al.)

Measurement of exhaled nitric oxide will be obtained prior to lung function, and will be obtained according to the American Thoracic Society Guidelines.(Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999) Exhaled nitric oxide concentrations will be measured using an FDA-approved handheld device that uses electrochemical technology to quantify FeNO levels (NIOX Mino System, Aerocrine, Sweden). This equipment is FDA-approved for clinical use in asthma management.

Lung function: Pre- and post-bronchodilator spirometry will be performed according to ATS guidelines.(Standardization of Spirometry, 1994 Update. American Thoracic Society) At least three reproducible flow-volume loops will be obtained using the portable Koko spirometer, after which albuterol will be administered via nebulizer. Approximately 10-15 minutes after completing the nebulized albuterol, spirometry will be repeated to obtain post-bronchodilator FVC, FEV1, FEF25-75, and PEF. By obtaining measures of pulmonary

function, we will be able to assess the relationship between changes in *Mus m* 1 exposure and pulmonary function, including the presence of reversible pulmonary obstruction, defined as a >12% increase in FEV1 after bronchodilator.

Saliva Collection: A 6-10cc sample of saliva will be obtained for analysis of biomarkers. Saliva will be collected at each clinic visit.

Buccal swabs for RNA, DNA extraction: The inside cheek will be swabbed to collect RNA AND DNA extraction. Approximately 6 swabs will be collected. Samples will be analyzed to determine if the environmental intervention was associated with epigenetic changes in candidate allergy-, asthma-, inflammation- or immune-related genes (DNA methylation and related RNA expression).

Venipuncture: A 20 ml venous blood sample will be obtained so that the serum can be used to measure total IgE and mouse allergen-specific IgG and IgE levels. As much as an additional 10mls of blood will be obtained for a complete blood count and differential, C reactive protein, and serum cytokines/chemokines and other inflammatory markers. At the Baltimore site only, an additional 20-30 ml blood sample may be obtained in participants ages 12-17 for studies of relationships between allergic inflammation and obesity and the immunologic characteristics of peripheral blood mononuclear cells.

Questionnaire: A questionnaire that captures medication use, asthma symptoms, and health care utilization will be administered by the research assistant at all clinic visits. The questionnaire will capture symptoms over a two week period of time, and medication use over a two week period of time. In addition, participants will bring all asthma medications with them to the clinic visit to ensure the collection of an accurate medication list. Asthma-related health care utilization will be captured for the preceding three month period of time, since health care utilization is a less frequent event than symptoms. Asthma control will be assessed using the Asthma Control Test (ACT) and quality of life will be assessed using questions from the CHSA. Adverse events will also be captured.

Because school age children and adolescents will be enrolled, we will administer the questionnaire to the primary caregiver of children 5-11 years, and to the study participant and his/her caregiver for adolescent study participants, age 12-17. Although previous studies have indicated concordance between the adolescent's and the caregiver's responses, we will collect data from both so that we have the ability to assess the concordance rate within our study population. For children 5-11 years, caregiver-reported symptoms will be

the primary source for this outcome data and for adolescents 12-17, adolescent-reported symptoms will be the primary source for this outcome data.

**Urine collection:** At the screening visit, a rapid urine cotinine semi-quantitative screening test, NicAlert, will be used to determine if a study participant  $\geq 12$  years of age is an active smoker. A NicAlert value  $\geq 3$  will exclude the participant from continuing with the study, but not from completing the baseline screening clinic visit. Urine will also be shipped to an outside laboratory for more quantitative measurement of urinary cotinine. These additional results obtained from an outside laboratory will not impact further continuation in the study, but will be used in analyses to assess second hand smoke exposure (and active smoking for the few participants who are active smokers who may not be identified by the NicAlert) as a confounder and effect modifier.

Female participants with a history of menstruation will have a urine pregnancy test done. Participants with a positive pregnancy test will not have skin prick testing or specific IgE testing, and will be ineligible to continue with the study.

Urine will also be used for measurement of F-isoprostane and other inflammatory markers at the screening visit.

All participants will first undergo confirmation of asthma symptom, medication, and health care utilization eligibility criteria. Participants who meet asthma severity, NicAlert criteria, are not pregnant and are allergic to mice (by skin prick test or mouse specific IgE) will be eligible for the home assessment visit. (See MOP)

If a subject cannot complete all CV1 activities at one visit, he/she may return within a week to complete the remainder of study activities.

### **7.1.2 Follow-up Clinic Visits**

The follow-up CVs, at 6 and 12 months, will include a questionnaire, pre- and post-bronchodilator spirometry, urine cotinine, saliva collection, buccal swabs and eNO. The 12 month CV will also include venipuncture for mouse-specific IgE and IgG levels, and at the Baltimore site only, an additional 20-30 ml blood sample may be obtained for studies of relationships between allergic inflammation and obesity and the immunologic characteristics of peripheral blood mononuclear cells in participants age 12-17. The same procedural methods described for the Screening CV will be used for these procedures at

the 6 and 12 month CV. The 6 and 12 month CVs will occur within a window of  $\pm 3$  weeks of the due date.

### 7.1.3 Telephone Calls (TC)

Telephone calls will occur at 3 and 9 months, within a window of  $\pm 3$  weeks of the due date. A brief questionnaire, modified from the baseline clinic visit questionnaire, will be administered to capture recent symptom history, asthma control, medication use, asthma-related health care use, and interval home environmental history.

### 7.1.4 Home Visit Procedures:

Home Assessment and Settled Dust Sample Collection will occur at the screening, 3, 6, 9, and 12 month home visits.

Home Assessment: A trained Research Assistant will conduct a home assessment to collect information regarding the home environment, including condition of dwelling, evidence of tobacco smoking, evidence of pets, and evidence of pests. A standardized home assessment form will be used to document the findings.

Dust sample collection: Dust samples will be collected from the bedroom and kitchen using standard procedures. Three dust samples will be collected, one from the bed, one from the bedroom floor, and one from the kitchen. Dust will be collected using a handheld vacuum cleaner according to procedures outlined in the Manual of Procedures. For randomized participants, dust samples will be analyzed for endotoxin, Mus m 1, Der p 1, Der f 1, Fel d 1, Can f 1, Rat n 1, and Bla g 2 content.

Buccal Swabs: Buccal Swabs, for DNA only, will be collected during the 3 and 9 month home visits if the study participant is at home.

The following procedures will be conducted at the screening, 6, and 12 month home visits only:

Air Monitoring for Particulate Matter and Allergen: At every other home visit (screening, 6, and 12 months), particulate matter monitoring will be conducted in the child's bedroom using integrated sampling direct-reading methods for approximately 72 hours according to procedures outlined in the Manual of Procedures. Integrated samples will be collected using constant airflow portable sampling pumps designed for quiet indoor operation. Air samples will

be shipped to the EBSC at Columbia University and extracted for allergen analysis according to methods described below. PM10 samples will be analyzed because previous research indicates that the bulk of airborne mouse allergen is contained on particles less than 10 µm in aerodynamic diameter.(Ohman, Jr. et al.) The PM10 sample may be analyzed for black carbon content and other components of PM10. Black carbon is a surrogate marker of diesel exposure and diesel exposure has been associated with asthma gene methylation. Black carbon or diesel exposure may confound the relationship between epigenetic changes and results of study intervention in the group of subjects assigned to receive IPM or assigned to receive Education.

PM10 At the screening, 6, and 12 month home visits, after the air monitoring equipment has collected airborne dust for approximately 72h, the air filters and equipment will be retrieved from the home.

If a participant qualifies to join the study and moves after randomization, the participant will continue to be followed in their new location. If they are in the IPM group and move after a home visit that triggers an IPM intervention every attempt will be made to get a baseline bed and bedroom floor dust sample prior to the IPM visit.

If a participant qualifies for the study and moves after Home Visit 1 but prior to randomization the Home Visit 1 will be repeated in the new location before randomization.

## 7.2 Laboratory Evaluations

### 7.2.1 Clinical and Research Laboratory Evaluations and Specimen Collection

#### CLINICAL SPECIMENS

Blood: Twenty milliliters of venous blood will be collected at the screening and 12 month clinic visits. The serum fractioned will be saved and shipped on dry ice in batches to the EBSC for the conduct of the IgE assays and shipment to Dr. Aalberse' laboratory for the mouse-specific IgG assays. At the screening visit only, up to an additional 10mls will be collected for a complete blood count with differential and C-reactive protein which will be measured in the clinical laboratories at each of the clinical sites. Also, at the screening visit and 12 month clinic visit at the Baltimore site only, an additional 20-30 ml may be collected for studies on peripheral blood mononuclear cells.

Mouse-specific Antibody Levels: Quantification of total IgE levels and mouse urine CAP-RASTs will be performed on serum using the ImmunoCap system (Pharmacia Diagnostics, Uppsala, Sweden). Total IgE will be quantified as a measure of atopy, and mouse urine CAP-RAST will provide a measure of allergic sensitization to mouse allergen, in addition to skin test sensitivity.

For participants with a negative mouse SPT at the screening clinic visit, serum samples will be shipped to the EBSC for mouse urine CAP-RAST. CAP-RAST results will be available within 2 weeks of the screening clinic visit so that eligibility for the study can be determined. SPT-, but specific IgE+ participants will be rescheduled for a screening clinic visit to complete all screening clinic visit procedures. Only mouse SPT+ or RAST+ participants will go on to have HV1 to determine if the exposure eligibility criterion is met (bed settled dust Mus m 1  $\geq 0.40$   $\mu\text{g/g}$ , or bedroom floor settled dust Mus m 1  $\geq 0.5$   $\mu\text{g/g}$ ). For potential study participants who cannot discontinue antihistamines, mouse-specific IgE levels can be used to determine sensitization status instead of skin prick testing.

Levels of mouse-specific IgG will be measured in serum samples in Dr. Rob Aalberse' laboratory using a solid phase antigen-binding assay as previously described.(Matsui et al.;Witteman et al.) Aliquots of serum will be shipped from the EBSC to Dr. Aalberse' laboratory.

#### Urine Cotinine

25 mls of urine will be collected at each clinic visit and shipped on dry ice to the EBSC. Urine will be shipped from the EBSC to an outside laboratory for measurement of urine cotinine. An aliquot of urine from the screening visit will be used for measurement of F-isoprostane and other inflammatory markers by an outside laboratory.

#### Saliva Collection

6-10mls of saliva will be collected at each clinic visit. The saliva will be aliquoted into whole saliva, supernatant, and pellets, with any remainder stored as whole saliva. Samples will be kept frozen and shipped on dry ice to Boston University for processing and will be used to measure inflammatory markers.

Our preliminary salivary inflammatory marker data from a similar study population suggests that phenotypic clusters of asthma can be identified using this biospecimen and the MAAIT study population will allow us to examine the role of salivary markers in phenotyping asthma and to determine the stability of the salivary inflammatory profile. Should the salivary inflammatory profile be stable, there will be compelling evidence that this approach may yield important information about inflammatory phenotypes. On the other hand, should the salivary inflammatory profiles change over time, then salivary markers may be

useful to track changes in inflammatory states among children/adolescents with asthma.

Therefore, salivary inflammatory markers will be measured to (1) assess the utility of salivary inflammatory profiling to identify novel asthma phenotypes, and, should the inflammatory profile change over time, (2) examine the impact of the mouse allergen targeted intervention on salivary inflammatory markers.

#### Buccal swabs for RNA and DNA

DNA methylation: Buccal cell DNA will be collected by swabbing of the inside of the cheek for approximately 1 minute. Individual swabs will be placed and stored in microcentrifuge tubes prefilled with Cell Lysis Solution. Samples will be frozen within 4 hours and then delivered to Dr. Rachel Miller's laboratory at Columbia University. Measures to preserve confidentiality will be maintained.

Buccal RNA Isolation: Gene expression using real time reverse transcription PCR will be done to determine whether methylation is associated with downstream molecular events, and the extent of methylation will be correlated with levels of RNA expression. Isohelix SK2 Buccal Swabs will be used to collect samples. Individual buccal swab samples will be placed in microcentrifuge tubes prefilled with RNeasy Protect® Cell Reagent. Total RNA is isolated using kits and standard materials in the lab.

#### Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72.

#### ENVIRONMENTAL SAMPLES

Allergen and Endotoxin Analyses: Allergen analyses will be conducted in the EBSC under the direction of Dr. Perzanowski.(Perzanowski et al.) Settled dust samples and air filters will be shipped from study sites to the EBSC on ice. Air filters will be collected after approximately 72 hours at the screening, 6, and 12 month home visits. Settled dust samples from the kitchen, bed and bedroom floor will be sieved and then extracted overnight at 4 degrees C. The settled



dust extracts will be analyzed for Mus m 1, Rat n 1, Der f 1, Der p 1, Bla g 2, Fel d 1, and Can f 1 by ELISA.(Wood et al.;Matsui et al.)

Settled dust endotoxin will be measured in Dr. Peter Thorne's laboratory at The University of Iowa. The kitchen floor settled dust samples will be shipped to Dr. Thorne's laboratory and assayed for endotoxin at varying dilutions using the kinetic *Limulus* amebocyte lysate (LAL) assay (BioWhittaker Inc., Walkersville, MD).

PM10 At the screening, 6, and 12 month home visits, after the air monitoring equipment has collected airborne dust for about 72h, the air filters and equipment will be retrieved from the home. When the PM10 air filters are retrieved from the home, elapsed time and flow rate will be recorded by the site study staff, and the filters will be shipped to Johns Hopkins where they will be weighed in a temperature and humidity controlled room. Once masses are obtained and recorded, filters will be shipped to the EBSC for allergen analysis. Soot-black carbon and other components of PM10 may be determined by using a multiwavelength integrating sphere method to achieve maximum specificity over a large range of ambient pollutant levels as published previously. This work will be conducted in Dr. Steven Chillrud's laboratory at Lamont-Doherty Earth Observatory of Columbia University.

## 7.2.2 Specimen Preparation, Handling and Shipping

### 7.2.2.1 Instructions for Specimen Storage

Serum will be stored at -20°C at each study site until shipment to the central clinical laboratory at Columbia, where it will be stored at -80 °C.

Urine will be stored at -20°C at each study site.

Saliva aliquots will be stored at -80°C at each site until shipped to Boston University.

Buccal swab samples will be stored at -20°C until shipped to Dr. Rachel Miller's laboratory at Columbia University.

Dust samples and air filters should be frozen at -20°C. Dust and air filter extracts will be stored at -20°C.

### 7.2.2.2 Specimen Shipment



Shipment procedures will be according to specific procedures outlined in the Manual of Procedures (MOP).

## 8. STUDY SCHEDULE

### 8.1 Screening Clinic Visit

The first activity at the screening clinic visit is the informed consent process. The study will have been reviewed with the parent/guardian prior to scheduling the screening visit. At the screening visit, the parent/guardian will be given ample time to read the consent form and ask questions. The consent designee on the study staff will also review the study procedures, risks, and potential benefits with both the parent/guardian and potential study participant, using age-appropriate language. If the parent/guardian and child would like to enroll in the study, the parent/guardian will sign and date the consent form, and the child will sign the assent form to indicate their willingness to participate. After obtaining written informed consent from the parent/guardian and assent from the child, the following procedures will occur at the screening visit:

1. Eligibility Questionnaire
2. Urine Sample Collection for: (1) NicAlert testing (ages 12+) and (2) pregnancy testing (female participants with history of menstruation)
3. Measurements
4. Skin Prick Testing
5. Saliva collection
6. Venipuncture
7. Questionnaire
8. Exhaled Nitric Oxide
9. Pre- and Post-bronchodilator spirometry
10. Buccal swabs for RNA and DNA

The eligibility questionnaire will be administered first to determine if the participant meets the clinical eligibility criteria. If the participant meets these eligibility criteria, a urine sample will be collected and rapid cotinine testing will be performed on the urine to screen for active smoking on children  $\geq 12$  years old and for pregnancy (for females who have a history of menstruation).

For participants who have met inclusion/exclusion (as determined by eligibility questionnaire) and are not pregnant or active smokers, all the screening clinic visit procedures will be completed. These include: skin prick testing, questionnaires, blood draw, exhaled nitric oxide, saliva collection, buccal swabs and pre- and post-bronchodilator spirometry and anthropometric measurements. For participants who had eligibility verified, are not pregnant, but are active smokers all screening clinic visit procedures will be completed except buccal swabs. (See MOP)

After completing all of the screening clinic visit procedures, participants who are not active smokers and meet the clinical and allergic sensitization eligibility criteria (SPT+, or mouse IgE positive) will have a home assessment visit within 3 weeks ( $\pm 1$  week).

## 8.2 Screening Home Visit

The screening home visit will include all study procedures since results of mouse allergen levels in dust samples collected from the home will not be available for 1-2 weeks following the home visit.

Screening home visit procedures will include:

- Completion of Home Assessment Form
  - Assessment of the presence of any major structural issues that could not be repaired with the procedures used in this study.
- Collection of settled dust samples from:
  - Kitchen
  - Bedroom floor
  - Bed
- The dust samples will be shipped to the EBSC at Columbia University for mouse allergen analysis.
- A return home visit will occur approximately 72h after the first home visit to collect the air sampling equipment.

## 8.3 Randomization

Randomization will occur within 3 weeks of completion of the screening home assessment visit, after results of mouse allergen analysis of bed dust sample has been completed by the EBSC at Columbia University. The mouse allergen concentration in the sample will be recorded on the Mouse Allergen Eligibility Form and will be faxed/and or emailed to the participant's study site. The mouse allergen concentration in the bed dust sample must be  $\geq 0.4 \mu\text{g/g}$  or the bedroom floor dust sample must contain  $\geq 0.5 \mu\text{g/g}$  of *Mus m 1* to meet exposure eligibility criteria. The coordinator will review the mouse allergen result and complete the eligibility checklist for the study participant, confirming that the participant meets all eligibility criteria. Once all eligibility criteria have been confirmed, then the study coordinator will use the Data Management System to randomize the study participant. Randomization will occur by site and with random blocks. The randomization scheme will be developed by the Data Coordinating Center (DCC) and embedded into the data management system so each site can randomize participants. Study staff will not have access to the randomization codes.

If the participant has been randomized to the Education Group, the study coordinator will schedule a home visit for Pest management education within  $2 \pm 3$  weeks.

If the participant has been randomized to the IPM Group, the study coordinator will schedule an IPM visit within  $2 \pm 3$  weeks.

## 8.4 Follow-up

Home Assessment Visits (HVs): HVs will occur at 3, 6, 9, and 12 months. The window period is within  $\pm 3$  weeks of the projected visit date. Home visit procedures include:

- Completion of Home Assessment Form and assessment of air filters, if present.
- Collection of settled dust samples from:
  - Kitchen
  - Bedroom floor
  - Bed
- Evidence of mouse infestation at the HV or reported by the study participant's parent/guardian at the HV will trigger an IPM module for participants randomized to the IPM Group.

The following procedures will occur only at the screening, 6, and 12 month home assessment visits:

Air monitoring equipment will be deployed for assessment of airborne PM10 and mouse allergen levels. A return home visit will occur approximately 72h after the first home visit to collect the air sampling equipment. The following procedures will occur only at the 3 and 9 month home assessment visits:

Buccal Swabs for DNA will be collected during the 3 and 9 month home visits if the study participant is at home.

Clinic Visits: Clinic visits will occur at 6 and 12 months. The window period is within  $\pm 3$  weeks of the projected visit date. Clinic visit procedures will include:

- Urine sample collection for cotinine testing by outside laboratory
- Questionnaire
- Exhaled Nitric Oxide
- Saliva collection
- Pre- and Post-bronchodilator spirometry
- Buccal swabs for RNA and DNA
- In addition to the above procedures, the 12 month clinic visit will also include venipuncture for assessment of mouse allergen-specific IgE and IgG levels.

### Telephone Calls

Telephone calls will occur at 3 and 9 months ( $\pm 3$  weeks) and will include a questionnaire designed to capture interval clinical history, current asthma control, report of mouse infestation, assessment of air filter use and functionality, and interval environmental history.

## **8.5 Early Termination Visit**

Participants may withdraw consent or be dropped from the study at any time. This includes, but is not limited to, voluntary withdrawal, noncompliance, lost to follow-up, and investigator discretion. Every effort will be made to contact the participant by phone to conduct a final telephone interview.

## **8.6 Unscheduled Visits**

There will not be unscheduled visits since the study will not be managing the study participant's asthma. Instead, documentation of the study participant's primary care provider will occur at the screening clinic visit and participants who do not identify a primary care provider will be referred to one. A participant who contacts study staff with acute health concerns will be referred to his/her primary care provider or the Emergency Department, depending on nursing or physician judgment.

## 9. ASSESSMENT OF SAFETY

### 9.1 Specification of Safety Parameters

Safety parameters will include clinical observation and administration of an unstructured questionnaire of the study participant and parent/guardian regarding any events during the course of enrollment in the study. Clinical observation will occur at each of the clinic visits and unstructured questioning will occur at each of the clinic visits and the home assessment visits.

### 9.2 Definition of an Adverse Event (AE)

An adverse event (AE) is any occurrence or worsening of an undesirable or unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease in a study participant, whether or not it is considered to be study-related. Any worsening of a pre-existing condition or illness is considered an adverse event.

An adverse event is considered unexpected when its nature or severity is not consistent with the descriptions in the protocol or consent form.

### 9.3 Definition of a Serious Adverse Event (SAE)

A serious adverse event (SAE) is defined as any adverse therapy experience occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution. This includes, but may not be limited to, any of the following events:

1. Death: A death occurring during the study or which comes to the attention of the investigator during the protocol-defined follow-up after the completion of the therapy whether or not considered treatment-related, must be reported.
2. Life-threatening: Any adverse therapy experience that places the subject or subjects, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that had it occurred in a more serious form, might have caused death).
3. Inpatient hospitalizations or prolongation of existing hospitalization.
4. Persistent or significant disability or incapacity
5. Congenital anomaly or birth defect.
6. An event that required intervention to prevent permanent impairment or damage.

## 9.4 Methods and Timing for Assessing, Recording, and Analyzing, Managing Safety Parameters

### 9.4.1 Methods and Timing for Assessment

At every clinic visit and telephone visit, study participants will be asked whether they have had any problems since their last study visit. Adverse events will be evaluated from the onset of the event until the time the event is resolved or medically stable. Adverse events may be discovered through any of these methods:

- Observing the participant
- Questioning the participant, which should be done in an objective manner
- Receiving an unsolicited complaint from the participant.

An adverse event form will be used for reporting all adverse events. An additional form will be required for serious adverse events to collect additional information.

#### 9.4.1.1 AE/SAE Grading and Relationship Assignment

Adverse events will be recorded and graded 1 to 5 according to the general grade definition below:

Grade 1 Mild Transient or mild discomforts, no or minimal medical intervention/therapy required, hospitalization not necessary (non-prescription or single use prescription therapy may be employed to relieve symptoms, e.g. acetaminophen or ibuprofen).

Grade 2 Moderate Mild to moderate limitation in activity; some assistance may be needed, no or minimal intervention/therapy required, hospitalization possible

Grade 3 Severe Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization possible.

Grade 4 Life-threatening Extreme limitation in activity, significant assistance required; significant medical therapy/intervention required, hospitalization or hospice care probable.

Grade 5 Death

The relationship to study procedures will also be recorded. Adverse events will be assessed as definitely related, probably related, or possibly related, or not related. An adverse event is considered related to the intervention or study procedures if there is a reasonable possibility that the adverse event may have been caused by the procedure.

1. **Definitely related:** An adverse event that follows a temporal sequence from administration of the procedure; follows a known response pattern to the procedure; is confirmed by improvement after stopping the procedure, and cannot be reasonably explained by a known characteristic of the participant's clinical state or by other therapies or interventions.
2. **Probably related:** An adverse event that follows a reasonable temporal sequence from administration of the procedure, is confirmed by improvement after stopping the procedure, and cannot be reasonable explained by the known characteristics of the participant's clinical state or other therapies.
3. **Possibly related:** An adverse event that follows a reasonable temporal sequence from administration of the procedure and follows a known response pattern to the procedure, but could have been produced by the participant's clinical state or by other therapies.
4. **Unlikely:** An adverse event whose temporal relationship to the study procedures makes a causal relationship improbable and in which other interventions or underlying disease provides plausible explanations.
5. **Not related:** An adverse event that does not follow a reasonable temporal sequence after administration of the procedure and most likely is explained by the participant's clinical disease state or other therapies.

#### **9.4.2 Recording and reporting of AEs**

An adverse event form will be used for reporting all adverse events. An additional form will be required for serious adverse events to collect additional information. Information that will be documented includes a brief description of the event, onset and duration of the event, severity/grade of the event, resolution status of the event, and relatedness to the study procedures. Any medical intervention will also be documented.

These forms will be faxed to the Clinical Coordinating and Administrative Core (CCAC) to the attention of the PI. For SAEs that must be reported in 24 hours to the CCAC, and, to the Data Management and Analysis Core (DMAC), the PI of the CCAC will be notified by email. The NIAID Medical Officer must receive notification of the SAE from the CCAC no later than 24 hours after the CCAC has received notification of the SAE and must receive the corresponding SAE report no more than 48 hours after the CCAC becomes aware of the event.

#### **9.4.3 Evaluation and Management of AEs and SAEs**

The study site investigator along with the PI of the Clinical Coordinating and Administrative Core (CCAC) will apply clinical judgment to determine whether an



adverse event is of sufficient severity to require that the subject be removed from treatment. If necessary, an investigator must suspend any study procedures and institute the necessary medical therapy to protect a subject from any immediate danger. AEs and SAEs will be followed until they resolve or become clinically stable.

Subsequent review by the SMC, IRB, and sponsor may suspend further study intervention at the site. The study sponsor and the SMC retain the authority to suspend additional enrollment and treatments for the entire study as applicable.

Although hospitalizations meet the SAE criteria as described in section 9.3, hospitalizations due to asthma exacerbations are ordinary, anticipated complications in this study population and only, exacerbations requiring hospitalization for 5 or more days or intensive care admission will be considered SAEs. For reporting purposes only, the date of onset of the exacerbations will be the date of hospital admission and the date of resolution will be the discharge date. For the purposes of this protocol, a pregnancy is considered an SAE and will be reported and followed until resolution of the pregnancy.

## 9.5 Reporting Procedures

Adverse event reports will be generated by the Data Management and Analysis Core (DMAC) on an ongoing basis and included in annual reports to the NIAID SMC. SAEs will be reported to the DMAC and the CCAC by the Study Coordinator and Site Investigator either within 24 hours or within 5-7 days as indicated below:

Any death or life-threatening SAE (Grade 4 or 5 SAE) must be reported to the CCAC within 24 hours. The following attributes must be assigned:

- Subject ID
- Description
- Date of onset and resolution (if known when initially reported)
- Severity
- Assessment of relatedness to the study procedures
- Action taken

The IRBs of both clinical sites must also be notified within 24 hours of the site's awareness of the event. SAEs must be reported in 24 hours to the CCAC, and the PI of the CCAC will be notified by email. The NIAID Medical Officer must receive notification of the SAE no later than 24 hours after the CCAC has received notification of the SAE and must receive the corresponding SAE report no more than 48 hours after the CCAC becomes aware of the event.

### **9.5.1 Specific Serious Adverse Event Requirements**

All serious adverse events will be recorded on the SAE-specific report form, reported as indicated in section 9.5, followed by a study physician until resolution or stabilization and reviewed for completeness and accuracy with the NIAID Medical Monitor prior to SAE closure.

## **9.6 Type and Duration of the Follow-up of Participants after Adverse Events**

Participants who have had AEs will be followed up until resolution or stabilization of the event, according to the site PI's judgment.

## **9.7 Halting Rules for the Protocol**

The protocol may be halted by the SMC, IRB, or Sponsor upon review of SAEs.

## **9.8 Stopping Rules for an Individual Participant/Cohort**

A study participant will be discontinued from further study intervention if any clinical adverse event, other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.

This includes an asthma hospitalization that results in intubation.

## **9.9 Premature Withdrawal of a Participant**

Voluntary withdrawal by the study participant may occur at any point during the course of the study. Every effort will be made to collect clinical and AE data via telephone.

## **9.10 Replacement of a Participant Who Discontinues Study Treatment**

Study participants who have completed an Education Visit or 1 IPM visit will not be replaced.

## 10. CLINICAL MONITORING STRUCTURE

### 10.1 Site Monitoring Plan

Site monitoring will happen in accordance with the Clinical Monitoring Plan (CMP) developed by NIAID for the MAAIT study. An external site monitor, Protocol Monitor/Clinical Research Associate, will be identified by each clinical site and approved by the NIAID Project Manager.

A medical monitor will be identified for each clinical site, Baltimore and Boston. The medical monitor will review safety reports on a regular basis and SAEs in real-time.

### 10.2 Safety Monitoring Plan

#### 10.2.1 Safety Review Plan by the SMC

The SMC will review any event as requested by the Investigators, CCAC, or NIAID. Memos summarizing scheduled or ad hoc SMC meetings will be drafted by the NIAID Medical Monitor and finalized after SMC's approval. Then memos will be forwarded to the CCAC for further distribution to IRBs and regulatory authorities as needed.

Further, the NIAID medical monitor will inform the SMC of any expedited SAEs at the same time as regulatory authorities.

## 11. STATISTICAL CONSIDERATIONS

### 11.1 Overview and Study Objectives

The primary objective of the MAAIT is to test the efficacy of IPM and room air filters in reducing asthma morbidity among mouse-sensitized children with asthma. The specific aims are:

- (1) To determine the efficacy of this mouse allergen-targeted environmental intervention to:
  - (a) Reduce asthma symptoms.
  - (b) Reduce rescue medication use, asthma exacerbations and asthma-related health care utilization.
  - (c) Improve physiologic and inflammatory markers of asthma disease activity.

- (2) To evaluate mouse-specific IgG and IgE as biomarkers of allergen exposure and asthma clinical status.
  - (a) To determine the impact of this intervention on mouse-specific IgG and IgE levels.
  - (b) To determine if changes in mouse-specific IgG and/or IgE are correlated with changes in settled dust and/or airborne mouse allergen measures.
  - (c) To determine if changes in mouse-specific IgG and/or IgE are associated with improvements in clinical, physiologic, and inflammatory outcomes.

## 11.2 Study Population

The study population will consist of children, ages 5-17 years, with moderate to severe asthma who are sensitized to mouse and have  $\geq 0.4\mu\text{g/g}$  of mouse allergen in bed dust or  $\geq 0.5\mu\text{g/g}$  of mouse allergen in bedroom floor dust samples. Study participants will be randomized to either the IPM Group or the Education Group and be followed over 12 months for collection of clinical outcome data and home exposure data.

## 11.3 Description of the Analyses

The MAAIT is a parallel group, randomized controlled trial designed to test the efficacy of an integrated pest management intervention on reducing household mouse allergen levels and improving asthma outcomes in mouse-sensitized children with asthma. The change in settled dust mouse allergen levels will be compared between the IPM and Education Groups to assess efficacy of allergen reduction. Asthma symptoms, rescue medication use, and health care utilization will be compared between IPM and Education Groups to assess efficacy of the intervention in reducing asthma symptoms and morbidity.

## 11.4 Measures to Minimize Bias

**Randomization:** Participants will be randomized within three weeks of the screening home visit in a 1:1 ratio using a statistical software package to generate random numbers. The randomization scheme will be developed by the DMAC. Participants at each site will be randomized independently of the other sites, and random blocks of 4 to 6 will be used to maintain similar enrollment into the two study groups at each site. There will be no stratification. Participants will be scheduled for an intervention visit or education visit, depending on the group to which the participant was randomized, within  $2 \pm 3$  weeks of randomization.

**Masking:** Masking in an environmental intervention study such as MAAIT is very difficult, if not impossible to accomplish. Although it may be possible to attempt to mask some study staff by increasing the number of research assistants at each site so that 1-2 RAs could be dedicated to collection of clinical outcome data, this would substantially

increase the budget. Moreover, even if this approach is taken, it is likely that families will divulge whether they have received IPM visits to the research staff that are collecting the clinical data. There are also several aspects of the study that will guard against bias that could result from having unmasked study staff. First, all laboratory assays will be conducted by laboratory technicians who will be masked to group assignments. Second, some of the clinical data that are collected, including exhaled nitric oxide and pre- and post- bronchodilator spirometry, are objective measurements that are less subject to influence by the study staff or study participants. Third, analyses will be conducted to determine if any improvements in clinical outcomes are associated specifically with decreases in home mouse allergen levels, not just to group assignment.

All laboratory studies (allergen ELISAs, allergen-specific IgE levels, mouse allergen-specific IgG levels, endotoxin levels, PM10 levels, black carbon levels, NO<sub>2</sub> levels, and urinary cotinine levels) will be performed in centralized laboratories in batches to minimize variability of the assay.

## **11.5 Appropriate Methods and Timing for Analyzing Outcome Measures**

The primary outcome is maximum days of asthma symptoms.

The primary clinical outcome measure, maximum number of days of symptoms in the preceding two weeks, will be assessed at the same time points as allergen levels (screening, 3, 6, 9, and 12 months) because this outcome variable has been shown to be related to current mouse allergen levels. Other clinical outcome variables that will be examined will include: days of rescue medication use, nights of nocturnal symptoms, days of exercise-related asthma symptoms, days of cough without a cold. These variables will be treated as count variables and analyzed accordingly. For key secondary outcome variables related to health care utilization that are rarer than symptom days, the mean number of each type of event will be analyzed; however, if these events are rare the variables will dichotomized. Prednisone bursts, unscheduled doctor visits, emergency department visits, and hospitalizations will also be examined.

### **Study Hypotheses**

(1) Reducing home mouse allergen exposure will be associated with improvement in asthma outcomes in mouse-sensitized children.

(2) Mouse-specific IgG and IgE levels will be correlated with direct measures of mouse allergen exposure.

## 11.6 Sample Size Considerations

The primary outcome variable is maximum days of asthma symptoms/2 weeks. Maximum symptom days are defined as the highest of three specific symptoms variables that capture days or nights of symptoms over the preceding 2 weeks: (1) nights of wakening, (2) days of slowed activity, and (3) days of wheezing, coughing or chest tightness. Thus, each subject has a value for the maximum symptoms days variable at each time point.

Assumptions for the sample size estimate for the symptom outcome are based on previous studies of inner-city children with asthma, (Matsui et al.; Morgan et al.) and on symptom data from an ongoing cohort study in Baltimore. The calculation is based on modeling the outcome as a Poisson distributed random variable allowing for overdispersion. Based on previous studies, we estimate that there will be a mean of 3.0 days of symptoms/ 2 weeks at screening, with a standard deviation of  $\pm 3.0$  (i.e. variance of 9), and that the intra-person correlation will be 0.3. Additional assumptions for the

Difference in Days of Symptoms/ 2 weeks	Power	N per intervention group	Total N	
0.5	0.9	306	612	
	0.8	228	456	
0.6	0.9	209	418	
	0.8	156	312	
0.7	0.9	151	302	
	0.8	113	226	

sample size calculation are that the primary analysis will be based on the final 3 follow-up visits at 6, 9, and 12 months, and equal sized intervention and control groups. Since days of symptoms/ 2 weeks is the primary clinical outcome variable, the sample size estimates have been

calculated so that the proposed study will be adequately powered to detect a difference in this outcome. At an  $\alpha=0.05$ , 252 study participants would give the study approximately 84% power to detect a difference of at least 0.7 days of symptoms between the two groups, and 70% power to detect a difference of 0.6 days between the two groups. The log relative risk between the treatment and control groups was the treatment effect upon which the sample size calculation was based and the variance of the estimate of the log relative risk was approximated using a Normal approximation (i.e. the "delta method"). The sample size was calculated accounting for the longitudinal (temporally correlated) nature of the data. (Table 5). Taking into account a projected 15% rate of early study exits that we have observed in previous studies, the Boston site will enroll as many as 125 participants and the Baltimore site will enroll as many as 250 to ensure that at least 300 participants will contribute to the primary endpoint of the study.

## 11.7 Maintenance of Trial Treatment Randomization Codes

The randomization scheme will be embedded in the data tracking system and access to the scheme will be restricted to the DMAC database programmer. In addition, once a

participant has been randomized, the group assignment will be locked. The randomization codes may only be broken at the request of the SMC and the minimum number of DMAC staff necessary will be unmasked in order to provide requested analyses to the SMC.

### **11.8 Participant Enrollment and Follow-Up**

Three hundred-fifty participants will be randomized, as many as 125 at the Boston site and as many as 250 at the Baltimore site. As many as 950 potential participants may need to be screened in order to identify 350 children who meet all eligibility criteria and can be randomized. Accrual will occur over a 36 month period, with an average of five participants/month randomized at each site. The duration of the study for each participant will be 13.5 months, so that total duration of enrollment and follow-up will last 49.5 months. Both study sites have a track record of excellent retention (>90%) of the target study population, and the sample size estimate has accounted for drop-outs, so that replacements of drop-outs will not be necessary.

Note: all participants exiting the study after randomization without having received even one intervention module (IPM or Education) will be replaced.

### **11.9 Safety Review**

The SMC will review accrual and adverse events annually provided by the DMAC.

### **11.10 Final Analysis Plan**

Analyses will be performed using a per-protocol (PP) dataset and an intent-to-treat (ITT) dataset. The primary analysis will be an ITT analysis. The ITT dataset will include all participants who are randomized, and the per-protocol (PP) dataset will include only participants who have completed the intervention visits per protocol (at least 75% of IPM visits for the IPM Group and or one education visit for the Education Group). For example, there is a maximum of 8 IPM visits if IPM is triggered at all home visits, so a subject for whom IPM is triggered at all visits will be included in the PP dataset if they received at least 6 of the IPM visits. For subjects requiring 6 IPM visits, at least 5 would need to be completed, and for subjects requiring 4 visits, at least 3 would need to be completed to be included in the PP dataset.

#### Analysis Plan:

Analyses will be performed using an intent-to-treat (ITT) dataset which will include all participants who are randomized.

Exploratory data analysis will include compilation of descriptive statistics to detect any outliers or discrepancies in data and to compare baseline characteristics and demographics between

IPM and Education Groups. Continuous variables will be summarized using means, medians, standard deviations, ranges, and interquartile ranges. Continuous variables will be analyzed using non-parametric approaches or will be transformed to meet assumptions of normality required for parametric statistics. Categorical variables will be tabulated. The percent of participants who complete the study, losses to follow-up, missed visits, and reasons for discontinuation will be presented.

The primary outcome for Aim #1 will be the participant's number of symptom days over a 2-week period (as described above), which will be collected every three months throughout the 12 month study period. The primary analysis will be conducted using the values of the primary outcome at the 6, 9, and 12 month follow-up visits. Secondary outcomes include: days of short-acting beta agonist use, days of slowed activity due to asthma, days of exercise-induced symptoms, days of cough without an upper respiratory infection, nights of waking due to asthma symptoms, oral corticosteroid bursts, and hospitalizations, emergency department visits, and unscheduled physician visits. Other secondary outcomes include FEV1/FVC, % change in FEV1 after albuterol, and eNO levels.

For the primary analysis of symptom-days we will use a log-linear model of the form

$$\log E[Y_{ij}] = b_0 + b_1 \text{trt}_i + b_2 \text{site}_i$$

where  $Y_{ij}$  is the number of symptom days for subject  $i$  on visit  $j$  ( $j = 1, 2, 3$ ),  $\text{trt}_i$  is the indicator of the intervention for subject  $i$ , and  $\text{site}_i$  is an indicator for the subject's site (Boston or Baltimore). The data will be assessed for the presence of over-dispersion (greater variability than expected under the Poisson model) and overdispersed Poisson models will be used where appropriate. To account for within-person correlations of the outcome, all models will be fit using a generalized estimate equations (GEE) approach using an exchangeable correlation structure.

In secondary analyses, we will adjust for factors that may affect responses to treatment such as the degree of exposure at baseline, the degree of sensitivity to mouse, number of other allergies, and asthma severity. For count outcomes we will use Poisson models and for continuous outcomes we will assume a normal distribution. Because hospitalizations, ED visits, unscheduled visits, and corticosteroid bursts are relatively infrequent events, we will assess the distribution of these variables and may decide to dichotomize the outcome as any vs. none and use logistic models.

Although there will likely be limited heterogeneity between sites, site differences will be assessed using exploratory data analysis. We will assess the heterogeneity in outcomes between sites using summary statistics and boxplots where a boxplot will be constructed for each site. We will look at the heterogeneity across sites relative to the heterogeneity within sites. Other potential confounders will be explored by examining any differences between the intervention and control groups and also assessing the association between the potential confounders at screening and at each study visit. In addition, potential confounders will be



included in the mean models to determine the impact the variables have on the relationship between group assignment and the outcome. Some specific confounders that will be carefully examined include study site, cockroach sensitization and exposure, medication use, changes in exposure to other indoor pollutants, including urine cotinine, and endotoxin levels, and season. Effect modifiers, including the degree of cockroach allergen reduction, will also be examined by stratifying analyses and creating an interaction term to include in the final models.

Missed study visits and drop-outs will occur, and if these events occur completely at random or at random, then the use of GEE in the final analyses will produce consistent estimates of the treatment effects and tests of these effects. (Diggle) However, dropouts may occur at random and be “informative” or “non-ignorable”. In this case, more sophisticated modeling approaches maybe needed to address this issue. Initially, we will conduct exploratory analyses to quantify the missingness and to discern possible differences between participants who drop out and those who remained in the study. Depending on the extent and information on the missingness in the data, we may utilize imputation techniques to assess the sensitivity of our finding using the complete data.

We will also explore whether there are clinical or exposure characteristics that predict a more robust response to the intervention. It is possible, for example, that children who are more highly sensitized to mouse may have the greatest benefit from the intervention. Other factors that may modify the effect of the intervention on outcomes include level of mouse allergen exposure, number of other allergic sensitivities, the combination of cat exposure+sensitization, and the combination of cockroach exposure+sensitization. Additional clinical characteristics that might affect responsiveness to the intervention include severity of asthma, lung function, and eNO concentrations. Analyses will be stratified by these potential response predictors and interaction terms will be included in models to determine if any of these factors predict responsiveness to the intervention.

For the Aim #2 outcomes, mouse-specific IgG and IgE levels, we will examine the distribution of these mouse-specific antibody levels between intervention and control groups at baseline and 12 months. The distributions of these mouse-specific IgG and IgE levels will be compared between the intervention and control groups at screening and 12 months to assess any differences. To account for the within-subject correlation associated with repeated outcome measures, we will estimate the heterogeneity in mouse-specific IgG and IgE levels at baseline and within a participant over time, and use a random effects model to account for these between and within-person/home relationships. Specifically, we will log-transform the response and fit the following Gaussian regression:

$$\log(\text{Mouse-specific IgG or IgE level}_{ij}) = b_{0i} + b_1 \text{trt} + b_2 \text{visit}_j + b_3 \text{trt} \times \text{visit}_j + a^T z_i + e_{ij}$$

where  $i$  denotes the subject,  $i = 1, 2, \dots, 350$ ,  $j$  denotes the visit ( $j = 0$  and  $12$ ), and  $z_i$  is a vector of potential confounders.  $b_{0i}$  is the random intercept that describes how the  $\log(\text{Mouse-specific IgG or IgE level})$  varies across homes at baseline, we assume  $b_{0i}$  is normally distributed with

mean  $b_0$  and variance  $\gamma^2$ .  $e_{ij}$  measures the heterogeneity in responses over time within subject  $i$ ; we assume  $e_{ij}$  is normal with mean 0 and variance  $\sigma^2$ . The estimate of the within-person correlation coefficient is calculated by taking  $\gamma^2 / (\gamma^2 + \sigma^2)$ .  $b_2$  represents how the log(Mouse-specific IgG or IgE level) changes over time for the Education group ( $\text{trt} = 0$ );  $b_3$  indicates the difference in how log(Mouse-specific IgG or IgE level) changes over time comparing the IPM to Education groups.

In addition to determining the effect of the intervention on mouse-specific IgG and IgE levels, we will also determine if changes in these antibody levels are correlated with changes in airborne and settled dust Mus m 1 levels (Aim 2b). We will construct scatterplots of  $\Delta\text{IgG}$  vs.  $\Delta\text{Mus m 1}$  for both IPM and Education Groups combined, and stratified by group. Pearson correlations (or Spearman, if data do not meet assumptions of normality) will be calculated for each of the pairs (IgG vs. airborne Mus m 1, IgG vs. settled dust, IgE vs. airborne Mus m 1, IgE vs. settled dust Mus m 1). Because of the relatively low frequency of health care utilization events, utilization over the previous 3 months for screening and 12 months will be dichotomized and the correlation between the change in utilization and IgG or IgE levels will be computed using a polyserial correlation measure.

In Aim 2c, we will determine if changes in mouse-specific IgG or IgE levels are correlated with improvements in the clinical, physiologic, and inflammatory outcomes. We will calculate differences between 12 month and baseline values for the continuous health outcome variables (FEV1, FEV1/FVC, eNO) and the symptom day and rescue medication use outcomes ( $\Delta$  symptom days,  $\Delta$  rescue medication use) and construct scatterplots of  $\Delta\text{IgG}$  vs.  $\Delta\text{FEV1}$  (and FEV1/FVC, eNO, symptom days) and calculate correlation coefficients.

Potential confounders will be explored by examining any differences between the intervention and control groups and also assessing the association between the potential confounders at baseline and at each study visit. In addition, potential confounders will be included in the random effects models to determine the impact the variables have on the relationship between group assignment and mouse-specific IgG and IgE levels. Some specific confounders that will be carefully examined include study site, presence of a cat in the home, and season. The outcomes of airborne and settled dust Mus m 1 levels will be analyzed using the same approach described for mouse-specific IgG and IgE levels.

Although MAAIT is not powered to statistically test for effect modification, the finding of effect modification with a biologic rationale would have important public health and scientific implications. Therefore, air filter use and the number of IPM visits will be examined as potential effect modifiers. Reported air filter use will first be compared to data documenting presence of an electric current to the air cleaner. If reported air filter use is moderately to strongly correlated with adherence data from the data loggers, an air filter adherence variable will be created, classifying participants by frequency of reported use. Changes in settled dust, airborne, mouse-specific IgG and IgE data will be compared across strata of air filter use using random effect models as specified above. Similarly, the distribution of number of IPM visits will be examined,

and strata of IPM visits will be created. It is possible that participants with a greater number of IPM visits will have a smaller reduction in mouse allergen levels as the number of IPM visits may be associated with a recalcitrant infestation. We will stratify by categories of numbers of IPM visits and examine the exposure outcomes across these strata. Similarly, it is possible that the efficacy of the intervention is modified by some participants in the Education Group adopting some interventions which may result in a reduction in mouse allergen levels. To account for this possibility, analyses will be stratified for adoption of these practices among the Education Group. It is also possible that moving may affect the efficacy of the intervention, so ad hoc sub-analyses may be conducted after excluding participants who have moved during the study to examine the potential impact of moving on the results of intervention.

Ad hoc analyses may also focus on differences in primary endpoints in specific groups of participants (including –but not only - participants from a specific study site and participants of Hispanic or non-Hispanic background)

## **12. QUALITY CONTROL AND QUALITY ASSURANCE**

Training of study staff will occur centrally prior to beginning recruitment. The training will include lecture, demonstration, and practice components to insure that all staff is fully trained. IPM technicians and IPM company management will complete training on the IPM protocol. Staff will complete certification to demonstrate acceptable levels of knowledge regarding each study component that they will be involved in performing.

The site PIs and study coordinators will be responsible for insuring that all procedures are performed according to the protocol. Periodic reviews of the procedures will be conducted by the study coordinator.

A MAAIT Manual of Procedures will be provided to all investigators and staff members. This manual will include detailed descriptions, including SOPs and case report forms, for each study activity or procedure.

Site visits will be conducted as outlined above and in the clinical monitoring plan to assess adherence to the protocol and progress of enrollment. Other areas of review will include data collection procedures, data entry, timeliness of form completion and data entry, and security measures for study data.

## **13. ETHICS/PROTECTION OF HUMAN SUBJECTS**

### **13.1 Institutional Review Board/Ethics Committee**

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate ethics review committee or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are placed into use. Only institutions holding a current US Federal-Wide Assurance issued by the Office for Human Research Protections (OHRP) may participate.

### **13.2 Informed Consent Process**

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the participants and their parents/guardians. Consent forms describing in detail the study interventions and procedures and risks are given to the participant and their parents/guardians. Written documentation of informed consent is required prior to starting the study. Consent forms will be IRB approved and the participant's parent/guardian will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant's parent or legal guardian and answer any questions that may arise. The participants and their

parents/guardians should have sufficient opportunity to discuss the study and process the information in the consent process prior to agreeing to participate. Spanish-speaking participants will be provided with consent documents in Spanish and the consent discussion will be facilitated or conducted by a translator. The participants and their parents/guardians may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

### **13.2.1 Assent Process**

Study participants will be 5-17 years of age and all will be engaged in a discussion of the study procedures and intervention and risks and potential benefits. Assent forms written in appropriate language for children will be reviewed with children and their parents/guardians and ample time will be provided to discuss the study procedures, intervention, risks and potential benefits. Written documentation of assent is required prior to starting the study.

## **13.3 Participant Confidentiality**

Following HIPAA guidelines, a participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and these numbers rather than names will be used to collect, store, and report participant information. Data reported in medical journals or scientific meetings will be presented in aggregate for participants as a whole. No individual participant will be identified in any way.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized hired party, without prior written approval of the sponsor.

## **13.4 Study Discontinuation**

Participants randomized to the Education Group will be offered two air filters, 1 set of mattress and pillow encasements, cleaning, and one IPM visit upon completion of the study.

## 14. DATA HANDLING AND RECORD KEEPING

### 14.1 Data Management Responsibilities

Data will be managed by a Data Management and Analysis Core (DMAC) under Dr. Matsui's direction. The DMAC consists of four full-time staff with programming, data management, and data analysis skills. Data analysis will be directed by Dr. Matsui with guidance from Dr. Roger Peng, Assistant Professor of Biostatistics. The DMAC currently maintains databases for 5 active studies, and is responsible for form development, data entry, data quality control, and statistical analyses. The DMAC also builds and maintains recruitment tracking databases. The DMAC will be responsible for coordinating all data collection and data management activities at all three study sites. In addition, the group will be responsible for conducting all data analyses.

Study recruitment and enrollment activities will be entered by site study staff into the recruitment and tracking database through a web-based portal. Data entered will include date of contact of study participant, eligibility status, dates due, and dates completed, for CVs, HVs, and TVs, etc. These data will be entered in real-time and staff will have the ability to generate automated reports of recruitment progress, study visit status, and retention data.

Questionnaires will be designed in Teleform®, which uses optical character recognition (OCR) technology, so that they can be scanned into the study database. Skin testing, spirometry and eNO will also be recorded on a scannable form. Documentation of acquisition of a biologic or environmental sample and its chain of custody will be documented on scannable forms. Forms will be scanned into a database system that will alert DMAC staff to any unclear data fields on these forms so that DMAC staff can verify the correct entry on the original data form. After scanning, the data will undergo a series of quality control checks designed to detect missing data points and invalid data points, such as incorrect dates.

Data that are not collected on scannable forms will be entered into a web-based data entry system. Allergen, IgE, endotoxin, and PM assay results will be entered into a web-based data entry system pre-populated with study ID and visit date and study activity using a graphic user interface. Entered data is verified, and missing data points or discrepancies in the data are flagged so that original documents can be verified.

Some data such as urine cotinine and mouse allergen-specific IgG data will be received on data spreadsheets. These data will be cleaned and checked for missing values and invalid data points.

All databases will be password protected and include user permissions. The databases will be maintained on a shared network drive or servers that are backed up twice daily.

Recruitment and study progress, and adverse event reports will be generated routinely by the DMAC to be shared with each of the study sites.

## **14.2 Data Capture Methods**

Data will be captured using a variety of capture methods which have been selected based on the type of data. Data that are easily collected on forms during clinic and home visits and telephone interactions will be captured on scannable forms and scanned into a database. Assay results generated by the EBSC will be directly inputted by laboratory staff into a web-based database that has been pre-populated with known variables such as study ID, date, and study activity type. Pre-populating tables will facilitate merging of data. Data generated by outside laboratories, such as Dr. Aalberse's laboratory, will be sent to the DMAC, and may be on data spreadsheets.

## **14.3 Types of Data**

The following types of data will be collected: environmental laboratory data (including environmental outcome data - mouse allergen levels), clinical laboratory data, clinical test data (allergy skin testing, lung function, exhaled nitric oxide), and clinical outcome data (symptom report, health care utilization, medications).

## **14.4 Source documents and Access to Source Data/Documents**

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP, regulatory and institutional requirements for the protection of confidentiality of participants. Study staff and investigators and site IRBs may access the records. As part of participating in a NIAID-sponsored, NIAID-affiliated study, each site will permit authorized representatives of the sponsor, NIAID, and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

## **14.5 Timing/Reports**

Adverse events and enrollment will be reviewed on an ongoing basis by the PI of the DMAC. Adverse events and enrollment reports will be generated for SMC review. Randomization codes will be broken and adverse events reported by treatment group at the request of the SMC.

## 14.6 Study Records Retention

Study documents must be maintained at the research center or a local storage facility for at least five years following the completion of the study. Study documents that must be retained include all Case Report Forms, laboratory reports, IRB approval documentation and related correspondence and signed informed consent/assent forms.

## 14.7 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with Good Clinical Practice (GCP ICH E6) Sections:

Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

Quality Assurance and Quality Control, section 5.1.1

Noncompliance, sections 5.20.1, and 5.20.2.

Site study coordinators and PIs are responsible for reporting any protocol deviations to the CCAC and regulatory bodies. Protocol deviations may also be identified during site monitoring visits. The DMAC will periodically run reports assessing: completion of all study procedures and timing of study activities and whether they are in window to identify protocol deviations.

Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.



## 15. PUBLICATION POLICY

All publications and presentations will be approved by a committee made up of the three PIs: Elizabeth Matsui, MD MHS; Matthew Perzanowski, PhD; and Wanda Phipatanakul, MD MS. Proposals for publications or presentations will be submitted to this committee for review and approval. If approved, a writing group will be assembled by the committee. The CCAC will coordinate and prioritize publication and presentation analyses and work with the writing group in developing appropriate analyses and presentation of results. The final product will be reviewed by the committee prior to publication and/or presentation.

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