



PROTOCOL FOR THE EUROPEAN COMMUNITY RESPIRATORY HEALTH SURVEY II



ECRHS II

Project Leaders:

Prof Peter Burney

Dr Deborah Jarvis

For further information:

www.ecrhs.org



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Department of Public Health Sciences
King's College London
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THE EUROPEAN COMMUNITY RESPIRATORY HEALTH SURVEY II

SUMMARY

The European Community Respiratory Health Survey II (ECRHS II) was developed as a follow-up study to The European Community Respiratory Health Survey I (ECRHS I). It was planned to assess the incidence and prognosis of allergy, allergic disease and low lung function in adults and look at change; describe the distribution of exposure to associated environmental risk factors; determine the extent to which exposure to these risk factors are attributable to disease; identify subgroups within the population most at risk and to collect and store blood samples suitable for DNA analysis that can be linked to health and environmental data in the future.

This protocol provides specific instructions on the use of questionnaires, lung function assessment, bronchial responsiveness tests, blood sample collection and dust sample collection. The main data collection sheets and questionnaires are provided in the appendices together with instructions and coding.

The protocol is published as a reference for those who are interested in the methods used in ECRHS II and also as an instruction manual for people wishing to collect comparable data.

MAIN PROTOCOL

Background

Diseases associated with allergy and poor lung function contribute substantially to morbidity and mortality in adults living in the developed world. It is well established that the prevalence of asthma, hayfever and eczema has increased substantially over the past 50 years, although the reasons for the increase are unknown. While it is recognised that the incidence of these conditions is greater in childhood than in adults some people will develop them for the first time during their adult life. Adults are exposed to a range of lifestyle and environmental factors that have been hypothesised to cause allergic disease and rapid loss of lung function, but the extent to which these exposures explain the incidence of these conditions in adults is largely unknown.

Varying estimates of asthma incidence

Previous studies provide varying estimates of the incidence of asthma in adults depending on the case definition and case ascertainment methods used.³⁻⁵ The geographical variation in the prevalence of asthma⁶ may be explained to some extent by variation in the incidence of adult asthma or variation in the prognosis of childhood asthma, but a standardised methodology is required to test these hypotheses. ECRHS aims to provide such a standardised methodology. Risk factors for adult onset asthma include gender,⁵ other allergic disease in particular rhinitis,^{4,5,7} sensitisation to allergen,^{4,8} exposure to hormonal replacement therapy⁹ and workplace exposures.¹⁰ The role of smoking remains contentious with some studies reporting an association with asthma and wheeze^{5,11} and others not observing this association.¹² The incidence of and risk factors for development of sensitisation to environmental allergen in adults is so far unknown although one report suggests that older adults who are 'atopic' are more likely to become 'non-atopic' than those who are younger.¹³ Remission of asthma symptoms has been reported in adults,^{5,14} it being more common in individuals with mild disease, but it is unknown whether any other environmental or lifestyle factors are important or whether loss or amelioration of symptoms reflects change in atopic status.

Lung function decline

Decline in lung function is an important predictor of overall mortality in the general population.¹⁵⁻¹⁷ Substantial variation in lung function has been reported across Europe¹⁸ but it is not known whether this reflects variation in lung development during childhood, maximal achieved FEV₁ or lung function decline in adulthood. Smoking, BR¹⁹ and atopy^{20;21} are associated with greater lung function decline but the effect of exposure to allergens is still uncertain.²² Public health interventions to reduce tobacco consumption and involuntary exposure to tobacco smoke remain the cornerstone of improving respiratory health and lung function within populations but other modifiable environmental exposures may also require control. There is emerging evidence that particulate pollution also contributes to lung function decline^{23;24} and further information on the strength of this association across differing populations is required to inform appropriate public health policy.

Sub-groups at risk

Some population sub-groups may be more vulnerable to the influence of environmental factors. Susceptibility may be related to genetic make-up or be defined by other physiological or clinical characteristics such as clinical disease, BR or atopy. For example, a substantial proportion of young adults who are sensitised to house dust mite have no overt clinical disease. It is uncertain whether as they grow older these individuals will experience greater decline in lung function or a greater risk of developing symptoms if they are chronically exposed to high levels of dust mite within their homes than those who are not sensitised.

Treatment

People with asthma who have low lung function are highly symptomatic and have poor quality of life. The aim of therapy, the mainstay of which is regular daily use of inhaled steroids, is to reduce symptoms and improve lung function by decreasing airway inflammation. These are effective treatments^{25; 26} but the extent to which their regular use prevents decline in FEV₁ over a prolonged period of time is unknown and the extent to which these treatments modify the influence of other risk factors such as smoking, BR and atopy²⁷ on disease progression is uncertain.

ECRHS I

From 1991-93, the European Community Respiratory Health Survey I (ECRHS I) studied more than 18000 young adults from more than 35 centres (predominantly, but not exclusively in Europe), collecting information on health status and a variety of factors known or hypothesised to be associated with the risk of developing asthma and atopy.^{1; 2} Many of the research teams who took part in this initial research effort agreed to continue their collaboration by re-examining participants in ECRHS II.

Insert map?

A random sample of at least 1500 men and 1500 women aged between 20 and 44 years was selected from appropriate local sampling frames. Each participant was sent a brief questionnaire asking about respiratory symptoms (Stage 1) and from those who responded, a random sample of 300 men and 300 women was selected to undergo a more detailed clinical examination (Stage 2). This included an extended interviewer administered questionnaire; blood tests for total IgE and specific IgE to house dust mite, grass, cat and *Cladosporium*; assessment of FEV₁ and FVC; and measurement of bronchial reactivity to methacholine. Participants who were unable to attend the testing centre were visited at home to have the questionnaire administered or were asked the questionnaire over the telephone. If they refused the questionnaire, an attempt was made to ascertain their smoking status.

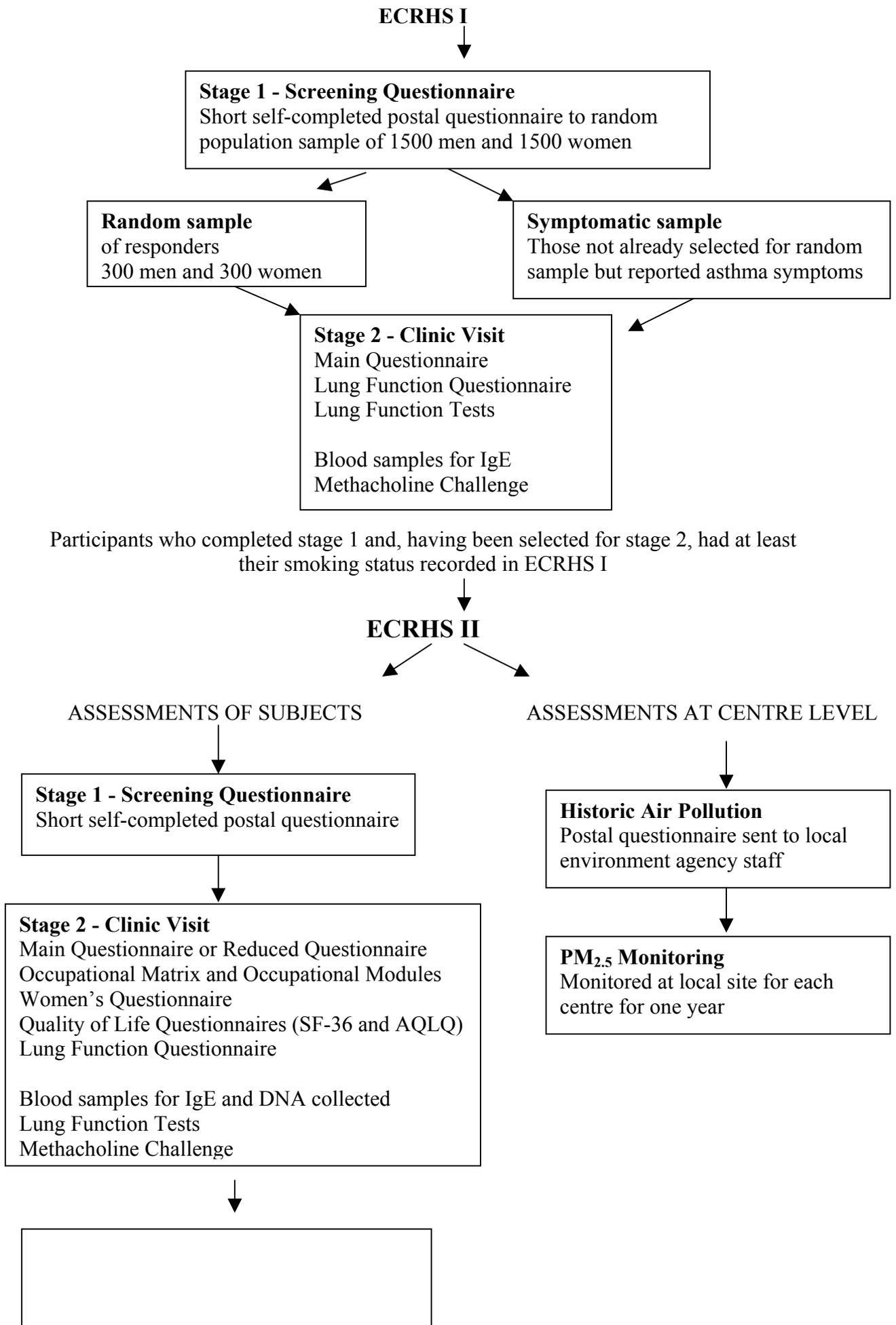
In addition to the random sample, a “symptomatic sample”, was also studied which comprised of participants who had not been selected from the random sample for the clinic assessment but who had reported symptoms of “waking with shortness of breath in the last 12 months” or “asthma attack in the last 12 months” or “taking asthma medication”. This “symptomatic sample” was invited to the testing centre to undergo the same tests as those that were performed on the random sample. In most centres, participants were asked to provide “named contacts” of friends and family likely to know their whereabouts should they move house over the next 10 years.

ECRHS I has been described in detail elsewhere^{1;2} and a review of main results from ECRHS I has recently been published.² ECRHS II is the follow-up study of this cohort.

The specific objectives of ECRHS II

- To determine the incidence and prognosis of allergy, allergic disease (asthma, hayfever and eczema) and low lung function in adults.
- To describe the distribution of exposure to known or suspected environmental risk factors associated with the incidence and prognosis of allergy, allergic disease and low lung function.
- To determine the risk attributable to chronic exposure to these environmental risk factors for the incidence and prognosis of allergy, allergic disease and low lung function.
- To identify subgroups within the population based on gender, prior disease status, bronchial responsiveness and genetic risk who may be more susceptible to these environmental risk factors and measure their excess risk.
- To establish a bank of blood samples suitable for DNA extraction taken from representative samples of the population that can be linked to health and environmental information.

Flow Chart of ECRHS



Study design

THE SAMPLE

All individuals who completed stage 2 of ECRHS I are eligible for ECRHS II.

The number of subjects fulfilling these criteria in each centre is given in Table 1.

Re-contacting the sample

Appropriate local measures should be taken to re-contact the sample.

A short self-completed questionnaire, identical to that used in stage 1 of ECRHS I, should be posted to the participant's address recorded in 1991-1993, with an accompanying letter asking them to take part in the next stage of the study. An appropriate number of mailings should be conducted to elicit maximum response by this means.

If no response is obtained and ethical permission has been granted, local databases likely to contain information on current address (e.g., population registers, health authority registers, electoral rolls) should be examined and participants contacted through mail, telephone or home visits. In addition the 'named contacts', identified during ECRHS I, could be approached for current contact details of participants.

Whatever method is used to re-contact the sample, the short questionnaire must be self-completed by all participants and response to this stage of the survey recorded **PRIOR** to any further tests that are conducted on the subject.

See Appendices A1 for the ECRHS II Screening Questionnaire and Instructions and A2 for Response Codes.

ASSESSMENT OF SUBJECTS

All participants who are contacted and have completed the short questionnaire are eligible to undergo further assessment in an appropriate testing centre.

Main Questionnaire

The main questionnaire is interviewer administered and should be conducted in a quiet room free of distractions. The questionnaire is made up of questions asking about exposures and health outcomes – many questions are similar to those that were asked in ECRHS I. The Reduced Questionnaire is made up of a selection of questions from the Main Questionnaire and can be used on the telephone making it useful if the subject refuses to come into the testing centre.

Questions include:

a) Symptoms and medical history

Respiratory symptoms are assessed using the same questions as in ECRHS I (taken from the bronchial symptoms questions of the International Union Against Tuberculosis and Lung Disease questionnaire²⁸⁻²⁹) and hay fever is assessed using questions used for ISAAC³⁰. Questions about eczema were based on the new working definition of eczema.³¹⁻³³ Severity of asthma is measured using questions based on the GINA guidelines classification of asthma severity.³⁴

b) Occupation

All occupations held since ECRHS I are recorded and coded using ISCO-88 codes.³⁵ In addition, those who report that they have worked as a cleaner, nurse or metal worker should be asked to complete a module giving more detail about their workplace activities. Participants reporting that they have been responsible for cleaning and washing in their home, that they have soldered or welded, or used disinfectants at home or at work since ECRHS I should also complete short modules asking for further detail of these activities. A Job Exposure Matrix³⁶ should be later applied to these occupations to determine the nature of exposures.

See Appendices C1 and C2 for Occupational Modules and Instructions and C3 for Occupational Matrix Coding Instructions. The Occupational Matrix is question 28 in the Main Questionnaire.

c) Home environment

Information on damp, mould, soft furnishings and exposure to domestic gas appliances is obtained using the same questions as in ECRHS I with additional questions on the frequency of use of gas cookers and ventilation in the kitchen included.

d) Air pollution

All participants are asked to rate their perceived exposure to air pollution using a visual analogue scale developed for SAPALDIA.³⁷

e) Medication and use of services

Usage of inhaled and oral drugs for the treatment of breathing problems is recorded using the same questions as in ECRHS I but further details of dosage and frequency is asked for. In particular, a detailed assessment of the use of steroid inhalers during the period of the follow-up is requested.

See Appendices B1 and B2 for Main Questionnaire and Instructions and Coding and Appendix B3 for Reduced Questionnaire.

Women's Questionnaire

Information on menstrual history, use of oral contraceptives and use of HRT since the last survey is collected using a questionnaire devised specifically for ECRHS II.

The same general rules for questionnaire administration applied as for ECRHS I.

See Appendices D1 and D2 for Women's Questionnaire and Coding.

Quality of Life Questionnaires

Participants should then be asked to complete the SF-36, a generic quality of life measure,³⁸ including two questions on chronic illness which ask if the subject has any long term limiting illness and lists eleven chronic illness conditions which the subject should respond whether they suffer from or not. Those who responded positively, in the short screening questionnaire, to having been woken by shortness of breath in the last twelve months or to having had an asthma attack in last twelve months or to currently taking medication should then be asked to complete the Juniper Asthma Quality of Life Questionnaire,³⁹ a disease specific quality of life questionnaire.

Allergy tests from serum IgE

Blood samples should be collected for the measurement of serum specific IgE and total IgE using the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). Serum samples should be stored at -20°C and then transferred to a centralised laboratory to be tested for specific IgE to house dust mite, grass, cat, *Cladosporium* and total IgE using the same method as in ECRHS I.

Insert picture of fieldworker taking blood sample?

Lung function testing

Baseline lung function measures (FEV₁ and FVC) should be taken using, wherever practically possible, the same equipment as ECRHS I. Lung function measures should be made in the sitting position with nose clips on and all subjects should attempt at least five forced expiratory manoeuvres. All manoeuvres deemed technically satisfactory should be recorded and, if less than two of the five are considered technically satisfactory, the participant should be allowed up to four further attempts. If after nine attempts two technically satisfactory manoeuvres have not been made, lung function testing should be abandoned. The procedure for lung function testing is detailed on page 20.

See Appendices E1 and E2 for lung Function Questionnaire and Instructions and Coding.

Insert picture of LF test in clinic?

Bronchial Responsiveness

In ECRHS I, centres were required to choose one of two dosing schedules for BR testing to methacholine (Provocholine®, Methapharm Inc.). The same method as used in the higher dosing schedule for ECRHS I should be adopted for ECRHS II. Firstly, participants should be asked to inhale a vapour of the diluent used to manufacture the methacholine solutions, the vapour being generated by a jet nebuliser set at an inhalation time of 1 second (Mefar, SRL, Italy). Depending on whether an individual reports symptoms in the long questionnaire or not, doubling (for those with symptoms) or quadrupling (for those without symptoms) doses of methacholine should then be inhaled until a 20% fall in post-diluent FEV1 is observed or the maximum cumulative dose of 2mg reached.

See Appendix E3 for Lung Function Data Sheets and Coding.

Blood samples for genetics

Blood samples should be collected into 3-10 ml EDTA tubes and frozen at the centre before transportation to a centralised laboratory in Munich. Samples will then be stored at -80°C until DNA is extracted using a commercially available kit (Puregene, Gentra Inc.). Following extraction, genomic DNA will be tested using agarose gels and a test PCR run with a TNFalpha polymorphism. DNA is quantified with the pico green dsDNA reagent (Molecular Probes). Finally, DNA will be distributed into 96 well master plates and adjusted to 300 µg/ml before being stored at -20°C for further analyses.

Indoor Environmental Assessment

For each centre, 200 homes should be visited for direct visual assessment of indoor characteristics such as type of flooring, presence of double-glazing, heating appliances and presence of damp using the Indoor Questionnaire. In addition, a sample of dust should be taken from the participant's mattress. An ALK dust collection filter (ALK-Abello, Horsholm, Denmark) should be attached to an Electrolux "Mondo" vacuum cleaner (1300 watts) and an area of 1 square metre vacuumed for a period of 2 minutes. An instruction video is available from the

ECRHS II Co-ordinating Centre. Dust samples should be sealed in a plastic bag, frozen for 24 hours at -20°C and then stored at room temperature, together with a silica gel desiccant. All samples should then be forwarded to the central laboratory for analysis of house dust mite allergen, using a standardised method.⁴²

Insert picture of fieldworker with dust sample?

See Appendices C1 and C2 for Indoor Questionnaire and Instructions.

ASSESSMENTS AT CENTRE LEVEL

Historical Air Pollution Data

Historical air pollution data should be collected to provide information on pollution levels over the follow-up period and to describe changes over time and variations across Europe. In collaboration with experts from the WHO European Centre for Environment and Health, Bilthoven / Bonn, questionnaires were developed and should be sent to staff working in government and local air quality monitoring agencies. The questionnaire requests all relevant information on local air quality obtained since ECRHS I with specific reference to arithmetic annual means and annual 95th or 98th percentiles for eight commonly measured pollutants, if available. Descriptive information regarding the location of the monitor(s) and the quality control level, according to international criteria, should also be asked for. From this, cities can be ranked by air pollution levels. The Historical Air Pollution Questionnaire is available from the ECRHS II Co-ordinating Centre.

PM_{2.5} assessment

A programme of PM_{2.5} monitoring should be carried out using a PM_{2.5} impactor for outdoor use (EPA Wins f/PQ167; BGI, USA). The same equipment has previously been used in the European exposure study EXPOLIS⁴⁰. Every month for a year, each centre should collect 6 samples on 7 days, over a two-week measuring period using a monitor installed within the original sampling area, resulting in a total of 84 days (72 filters) per centre to derive an annual mean. Details of this protocol will be presented elsewhere but, in summary, a standardised protocol has been developed, predominantly by the University of Basel study partners, who are responsible for pre-

and postweighing all Teflon filters in a centralised laboratory. The PM_{2.5} filters will then be analysed for selected chemical elements using x-ray spectrometry by the same research team.⁴¹

Insert picture of Pm2.5 monitor?

The Standard Operating Procedure for PM_{2.5} monitoring is available from the ECRHS II Co-ordinating Centre.

TRAINING AND QUALITY CONTROL

Local training of fieldworkers, using the local language, should be conducted using a standardised protocol for questionnaire and health assessment. Fieldworkers from more than one centre, but within the same language group, can be trained alongside each other. Adherence to the protocol should be assessed through a quality control visit by a member of the co-ordinating centre to at least one centre in each region, with subsequent visits being conducted by a nominated member of this centre to other centres in the region. Where deviations from the protocol are observed they should be rectified to ensure standardisation across the study.

The statistical method adopted by ECRHS I for analysing bronchial responsiveness, to a large extent, takes account of variation in performance of the equipment used for delivery of methacholine vapour.⁴³ However, nebulisers used in the study should be initially calibrated in Melbourne⁴⁴ to ensure that they all have similar aerosol output. In addition, monthly checks of the pressure of the Mefar dosimeter should be carried out in each centre and records sent to the Co-ordinating Centre.

As well as the central quality control initiatives, each centre and region should adopt appropriate local and regional strategies for maintaining high quality data and standardisation. For example, in Spain, interviewer technique was assessed by recording one out of ten interviews in all five participating centres. Tapes were then reviewed by staff in one centre to check for standardisation and good interviewer technique.

Training workshops for both indoor environmental assessment and PM_{2.5} assessment should be held. For the indoor protocol, a video demonstrating the method of collection of dust samples was developed and is available from the Co-ordinating Centre. For PM_{2.5} assessment, a one day workshop where staff are shown how to set up and maintain equipment should take place. Further quality control procedures should be carried out for this part of the protocol including copying downloads of pump performance to the outdoor pollution centre following completion of measures, site visits and remote quality control strategies.

LABORATORIES FOR ECRHS II

- All blood samples are analysed for IgE at King's College London, UK
- All dust samples are analysed for house dust mite allergen at King's College London, UK
- All extraction of DNA is carried out at the University of Munich, Germany
- All PM_{2.5} analyses are carried out at the University of Basel, Switzerland

Organisational structure

The study is managed through a single Steering Committee and several Working Groups (Outdoor Air Pollution, Occupation, Indoor Environment, Gender, Genetics, Early Life, Quality of Life, Therapy and Economics and Diet). The chair(s) of each Working Group is automatically a member of the Steering Committee and other researchers were invited to become members as appropriate. Working Groups, comprising both junior and senior researchers directly involved in the project, were responsible for protocol development to ensure collection of information pertinent to their specific area and is responsible for the analysis and publication of results within their area. The integration of the large number of researchers, research teams and Working Groups is facilitated through the Co-ordinating Centre of the study which also acts as a central resource for all queries from participating centres, facilitates the financial management of the study and develops the web-site for dissemination of the study (www.ecrhs.org).

CO-ORDINATING CENTRE

Project Leader: P Burney; Statistician: S Chinn; Principal Investigator: D Jarvis; Principal Investigator: C Luczynska; Project Co-ordinator: J Knox; Assistant Statistician: J Potts; Data Manager: S Arinze.

STEERING COMMITTEE

Professor Josep M Antó, Institut Municipal d'Investigació Mèdica, Universitat Pompeu Fabra (IMIM-UPF); Professor Peter Burney, King's College London (Project Leader); Dr Isa Cerveri, University of Pavia; Miss Sue Chinn, King's College London; Professor Roberto de Marco, University of Verona; Dr Thorarinn Gislason, Iceland University Hospital; Dr Joachim Heinrich, GSF – Institute of Epidemiology; Assoc Professor Christer Janson, Uppsala University; Dr Deborah Jarvis, King's College London; Miss Jill Knox, King's College London; Dr Nino Kuenzli, University of Basel; Dr Bénédicte Leynaert, Institut National de la Santé et de la Recherche Médicale (INSERM); Dr Christina Luczynska, King's College London; Dr Françoise Neukirch, Institut National de la Santé et de la Recherche Médicale (INSERM); Dr J Schouten, University of Groningen; Dr Jordi Sunyer, Institut Municipal d'Investigació Mèdica, Universitat Pompeu Fabra (IMIM-UPF); Dr Cecílie

Svanes, University of Bergen; Professor Paul Vermeire, University of Antwerp; Dr
Matthias Wjst, GSF – Institute of Epidemiology

PRINCIPAL INVESTIGATORS AND SENIOR SCIENTIFIC TEAM

Belgium: South Antwerp & Antwerp City (P Vermeire, J Weyler, M Van Sprundel, R Van Grieken, V Nelen). **Estonia:** Tartu (R Jogi, A Soon). **France:** Paris (F Neukirch, B Leynaert, R Liard, M Zureik), Grenoble (I Pin, J Ferran-Quentin). **Germany:** Erfurt (J Heinrich, M Wjst, C Frye, I Meyer). **Iceland:** Reykjavik (T Gislason, E Bjornsson, D Gislason, T Blondal, A Karlsdottir). **Italy:** Turin (M Bugiani, P Piccioni, E Caria, A Carosso, E Migliore, G Castiglioni), Verona (R de Marco, G Verlato, E Zanolin, S Accordini, A Poli, V Lo Cascio, M Ferrari), Pavia (A Marinoni, S Villani, M Ponzio). **Netherlands:** Groningen & Geleen (J Schouten, M Kerkhof). **Norway:** Bergen (A Gulsvik, E Omenaas, C Svanes, B Laerum). **Spain:** Barcelona (JM Antó, J Sunyer, M Kogevinas, JP Zock, X Basagana, A Jaen, F Burgos), Huelva (J Maldonado, A Pereira, JL Sanchez), Albacete (J Martinez-Moratalla Rovira, E Almar), Galdakao (N Muniozgueren, I Urritia), Oviedo (F Payo). **Sweden:** Uppsala (C Janson, G Boman, D Norback, M Gunnbjornsdottir), Goteborg (K Toren, L Lillienberg, AC Olin, B Balder, A Pfeifer-Nilsson, R Sundberg), Umea (E Norrman, M Soderberg, K Franklin, B Lundback, B Forsberg, L Nystrom). **Switzerland:** Basel (N Kuenzli, B Dibbert, M Hazenkamp, M Brutsche, U Ackermann-Liebrich). **United Kingdom:** Norwich (D Jarvis, B Harrison), Ipswich (D Jarvis, R Hall, D Seaton)

Centres taking part at their own expense

Australia: Melbourne (M Abramson, R Woods, EH Walters, F Thien). **France:** Bordeaux (A Taytard, C Raheison), Montpellier (J Bousquet, P Demoly). **Germany:** Hamburg (K Richter). **USA:** Portland (M Osborne, S Buist, W Vollmer, L Johnson).

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Lung Association of Oregon, Northwest Health Foundation, Collins Foundation, Merck Pharmaceutical; **Reykjavik**: Icelandic Research Council, Icelandic University Hospital Fund; **Tartu**: Estonian Science Foundation; **Turin**: ASL 4 Regione Piemonte (Italy), AO CTO/ICORMA Regione Piemonte (Italy), Ministero dell'Università e della Ricerca Scientifica (Italy), Glaxo Wellcome spa (Verona, Italy); **Umeå**: Swedish Heart Lung Foundation, Swedish Foundation for Health Care Sciences & Allergy Research, Swedish Asthma & Allergy Foundation, Swedish Cancer & Allergy Foundation; **Uppsala**: Swedish Heart Lung Foundation, Swedish Foundation for Health Care Sciences & Allergy Research, Swedish Asthma & Allergy Foundation, Swedish Cancer & Allergy Foundation; **Verona**: University of Verona; Italian Ministry of University and Scientific and Technological Research (MURST); Glaxo-SmithKline Italy.

Table I - Number of eligible participants per centre in ECRHS II as defined by number of responders to stage II of ECRHS I

Country	Centre	Number of Eligible Participants from Random Sample	Number of Eligible Participants from Symptomatic Sample	Total Number of Eligible Participants
ICELAND	Reykjavik	564	84	648
SWEDEN	Umea	552	156	708
	Uppsala	622	201	823
	Goteborg	682	184	866
ESTONIA	Tartu	431	127	558
NORWAY	Bergen\$	835	0	835
UK	Norwich	473	108	581
	Ipswich	448	111	559
GERMANY	Hamburg*\$	1252	0	1252
	Erfurt\$	731	0	731
NETHERLANDS	Groningen\$	380	0	380
	Geleen\$	415	0	415
	Bergen-op-zoom+\$	452	0	452
BELGIUM	Antwerp City	564	87	651
	South Antwerp	558	76	634
SWITZERLAND	Basel	853	149	1002
FRANCE	Paris\$	652	0	652
	Bordeaux*\$	544	0	544
	Grenoble	473	49	522
	Montpellier*\$	456	0	456
SPAIN	Oviedo	357	167	524
	Galdakao	486	106	592
	Barcelona	393	123	516
	Albacete	435	191	626
	Huelva	271	132	403
ITALY	Verona	340	18	358
	Pavia\$	310	0	310
	Turin	244	111	355
USA	Portland*	723	116	839
AUSTRALIA	Melbourne*	669	207	876
TOTAL		16165	2503	18668

* Non EU funded centres, taking part at their own expense

+ At time of writing it is unlikely that this centre will provide data for ECRHS II

\$ Centres who, due to limited resources, only studied a representative sample of responders to stage I and therefore have no 'symptomatic sample'

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LUNG FUNCTION TESTS

CRITERIA FOR TESTING

Criteria for baseline spirometry

The purpose of baseline spirometry is to record an accurate Forced Expiratory Volume in one second (FEV₁) and Forced Vital Capacity (FVC) from every subject who attends the testing centre.

ACCEPTANCE CRITERIA:

Any subject who is able to attend the testing centre.

EXCLUSION CRITERIA:

If the subject smokes: Lung function testing should be carried out at least one hour after the last cigarette has been smoked.

If the subject has used an inhaler: Lung function testing should be carried out at least one hour after the use of any inhaler.

If the subject has used an inhaler that is not a beta-2-agonist or an anticholinergic inhaler in the last one to four hours: Lung function testing should be carried out and the data recorded.

If the subject has used an inhaler that is a long acting Beta-2-agonist in the last 8 hours: If the subject is willing to come back at another time when they have not taken their long acting Beta-2-agonist, another appointment should be made. HOWEVER – this may be difficult for them to do, in which case, testing should proceed and medication used recorded.

If the subject has used an inhaler that is a beta-2-agonist or an anticholinergic inhaler in the last one to four hours: If the subject is willing to come back another time for lung function

testing, another appointment should be made. If the subject is unable or reluctant to return another time, testing should proceed and the medication used recorded.

If the subject has taken an oral beta-2-agonist or an oral theophylline or an oral antimuscarinic within the last eight hours: If the subject is willing to come back another time for lung function testing, another appointment should be made. If the subject is unable or reluctant to return another time, testing should proceed and the medication used recorded.

If the subject has had a respiratory tract infection in the last three weeks: Another appointment should be made unless the subject is unwilling to come back, in which case testing should continue. The number of days elapsed since the end of the respiratory infection should be recorded.

If, after a total of nine attempts, a subject is unable to produce a technically satisfactory manoeuvre, no FEV₁ or FVC should be recorded.

Predicted FEV₁ values

Normal FEV₁ values should be calculated using the following equations:

Males: $4.30 H - 0.029 A - 2.49$

Females: $3.95 H - 0.025 A - 2.60$

where

H = height in metres

A = age in years

Criteria for methacholine challenge

The aim of methacholine challenge is for subjects to inhale increasing concentrations of methacholine solutions and to monitor any change in FEV₁ by repeated spirometric testing.

ACCEPTANCE CRITERIA: Any subject who fulfils all three of the following criteria should be accepted:

- 1) has been able to perform at least 2 technically satisfactory manoeuvres during baseline spirometry
- 2) has signed a consent form for methacholine challenge
- 3) is not in the categories for exclusion (see below)

EXCLUSION CRITERIA: Any subject who fulfils any one of the following criteria should be excluded from methacholine challenge:

- 1) has had a heart attack in the last three months
- 2) has any heart disease for which he/she is taking medication
- 3) has epilepsy for which he/she is taking medication
- 4) is pregnant
- 5) is breast feeding
- 6) is taking a beta-blocker for any reason (including eye drops)

These criteria will be assessed by the Lung Function Questionnaire (*see Appendix E1*).

In addition, any subject who fulfils either of the following should be excluded:

- 7) has an FEV₁ less than 70% of the predicted value,
- 8) has an FEV₁ less than 1.5 litres.

FEV₁ is the maximum assessed during the baseline spirometry.

Criteria for bronchodilator challenge

The FEV₁ and FVC should be measured following the administration of 400µg salbutamol by metered dose inhaler (MDI) via a volumatic spacer.

ACCEPTANCE CRITERIA: Any subject who fulfils all of the following criteria should be accepted:

- 1) has produced technically satisfactory FEV₁ and FVC manoeuvres
- 2) refuses to undergo or is excluded from methacholine challenge
- 3) has signed a consent form for bronchodilator challenge
- 4) is not excluded by the following exclusion criteria

EXCLUSION CRITERIA: Any subject who fulfils any one of the following criteria should be excluded:

- 1) has had a heart attack in the last three months
- 2) has any heart disease for which he/she is taking medication
- 3) has epilepsy for which he/she is taking medication
- 4) is pregnant
- 5) is breast feeding
- 6) is taking a beta-blocker for any reason (including eye drops)

These conditions will be assessed by the Lung Function Questionnaire (*see Appendix E1*).

Making the appointment for testing

Ideally, lung function testing should be performed:

- 1) more than four hours after the use of a beta-2-agonist or anticholinergic inhaler
- 2) more than eight hours after inhaled long acting beta-2-agonist, oral beta-2-agonist or theophylline or oral antimuscarinic

When the appointment for lung function testing is made the fieldworker should determine if the subject is taking any of the following medications:

- 1) beta-2-agonist inhaler (short or long acting)
- 2) anticholinergic inhaler
- 3) oral beta-2-agonist
- 4) oral theophylline
- 5) oral antimuscarinic

If the subject is taking any of these medications (or any other inhaler) an appointment time should be agreed that will cause the least disruption to the subject's normal dosing schedule.

One simple way of ensuring compliance with these instructions is to:

- 1) avoid early morning appointments for those using inhalers
- 2) fix a time for an appointment and then ask the subject to take their inhalers four hours before and oral medication eight hours before testing. Ask them to avoid taking their long acting beta-2-agonist if possible

The fieldworker should ensure that the subject has not had a respiratory tract infection in the three weeks prior to testing and should advise the subject not to smoke for one hour prior to coming to the testing centre. A letter should be sent to the subject explaining this

Subjects who have not followed guidelines

Those who have had a cigarette in the last hour should have the lung function test delayed until one hour has elapsed (most subjects will be in the centre for at least one hour).

Those who have had an inhaler in the last four hours or oral medication (or long acting beta-2-agonist) in the last eight hours may fall into one or more of the following categories:

- 1) misunderstood the instructions
- 2) forgot the instructions
- 3) ignored the instructions
- 4) may have symptoms too severe to follow the instructions

Lung function testing may still be carried out unless the subject is excluded for other reasons, and recent medication should be noted in the Lung Function Questionnaire.

THE FORCED EXPIRATORY MANOEUVRE

General guidelines

All forced expiratory manoeuvres should be performed:

- 1) sitting, legs uncrossed
- 2) with noseclip on
- 3) using a plastic or cardboard mouthpiece without teethgrips
- 4) tight clothing should be loosened

Two types of forced expiratory manoeuvre should be used in this protocol:

- During baseline spirometry and bronchodilator challenge FVC will be measured and all subjects must exhale fully
- During methacholine challenge only the FEV₁ needs to be recorded and the technician may interrupt the exhalation when this has been achieved

A technically unsatisfactory manoeuvre (FEV₁ or FVC) is defined as:

- 1) an unsatisfactory start of expiration characterised by excessive hesitation or false start
- 2) coughing during the first second of the manoeuvre, thereby affecting the measured FEV₁ value, or any cough that interferes with the accurate measurement of FVC
- 3) Valsalva Manoeuvre (glottis closure)
- 4) a leak in the system or around the mouthpiece
- 5) an obstructed mouthpiece, e.g. the tongue in front of the mouthpiece.

Manoeuvres which have these faults are technically unsatisfactory and should be rejected as failed attempts.

Evidence of poor compliance is shown by:

- 1) greater than 200mL (NB in ERCHS I this was 5%, this has been changed in line with current ATS criteria) variation in FEV₁ between blows
- 2) greater than 150 mL or 5% FVC back-extrapolated volume
- 3) peak expiratory flow that is less than 85% of the best record
- 4) expiratory time that is less than six seconds

If these features are noted technicians should encourage the subject to produce a better reading but the blows should not be excluded as failed attempts on these criteria alone.

A manoeuvre should only be rejected as a failed attempt if it is 'technically unsatisfactory'. Manoeuvres with evidence of 'poor compliance' only should not be rejected.

The above protocol is consistent with current ATS guidelines (Am J Respir Crit Care Med 1995;152:1107-1136). These state that 'The only criterion for unacceptable performance is fewer than two acceptable curves. No spirogram should be rejected solely on the basis of its poor reproducibility.....elimination of data from subjects who fail to meet ATS reproducibility criteria may result in population bias by excluding subjects who have abnormal lung function'

Instructions to subjects

Some of the subjects will never have used any form of lung function testing equipment before and others will be very familiar with it.

Technicians should explain to the subject that the aim of the test is to find out how much air can be blown out of the lungs and how forcefully it can be blown out.

This can be done by asking the subject to follow these steps:

- 1) Take in as deep breath as possible
- 2) Place the mouthpiece in his/her mouth
- 3) Close his/her lips tightly around the mouthpiece
- 4) Blast or blow through the mouthpiece into the spirometer, blowing air out as hard, fast, smoothly and completely as possible

The subject should continue to push out air actively for as long as possible (FVC manoeuvre) or until the technician tells him/her to stop (FEV₁ manoeuvre). During this time the technician must offer positive encouragement to push or squeeze out more air.

Baseline spirometry

- 1) Ensure that it is appropriate to perform lung function testing
- 2) Demonstrate the manoeuvre to all subjects at least once (more often if he/she appears uncertain).
- 3) Ask the subject to carry out five FVC manoeuvres
- 4) Record the FEV₁ and FVC and Peak Expiratory Flow (in litres per second) from at least two and up to five technically satisfactory manoeuvres
- 5) If the subject has failed to produce two technically satisfactory manoeuvres after five attempts, the technician should show them again how to conduct the manoeuvre and allow them four more attempts
- 6) Any subject who is unable to produce two technically satisfactory manoeuvres after nine attempts should not be tested further and no FEV₁ / FVC data should be recorded
- 7) The number of rejected attempts should be recorded as appropriate on the Lung Function Data Collection Sheet

Methacholine challenge

During methacholine challenge the subject may need to perform 30 or more expiratory manoeuvres and, to minimise exhaustion, the forced expiration will be abandoned each time after one second when the FEV₁ has been recorded.

- 1) Two minutes after inhalation from the dosimeter up to five attempts should be made to record an FEV₁

- 2) As soon as two technically satisfactory manoeuvres have been achieved these readings are recorded. The next dose can be given as soon as possible after the completion of these measurements
- 3) Further testing should be abandoned if the subject is unable to produce two technically satisfactory manoeuvres within five attempts

If a reversal of bronchoconstriction needs to be carried out then the procedure is the same as the bronchodilator challenge.

Bronchodilator challenge

A bronchodilator challenge will be given to those who do not undergo methacholine challenge. Any subject who has more than a 10% fall in FEV₁ from baseline during the methacholine challenge test should have their bronchoconstriction reversed at the end of the test and before leaving the test centre, by the same method.

The salbutamol inhaler should be shaken and inserted into the volumatic. One puff should be activated and the subject asked to place their lips around the volumatic and to inhale and exhale five times. The salbutamol inhaler should be activated again and five inhalations/exhalations performed. This should be repeated two more times so that a total of 400µg of salbutamol has been delivered. Subjects who are known asthmatics and familiar with volumatic usage can self-administer this dose.

The FEV₁ and FVC are measured 10 minutes after the administration of bronchodilator. During the bronchodilator challenge FVC manoeuvres will be used. Up to nine attempts may be made to obtain two technically satisfactory recordings after the inhalation of bronchodilator.

THE METHACHOLINE SOLUTIONS

Source and supply

Methacholine (Provocholine) should be obtained from Methapharm.

The Diluent

Saline buffered with phosphate to obtain physiological pH can be used as a diluent. Phenol must not be used as a preservative because of concerns regarding its safety. Citric acid/citrate buffer must not be used. Preservatives should be avoided.

Session number and order in session

Each time the nebulisers are filled with fresh methacholine solution a new session of testing is said to have started. Each session should be sequentially numbered. Each challenge within each testing session should also be sequentially numbered and recorded on the Lung Function Data Collection Sheet (*see Appendix E3*).

At the beginning of a session all nebulisers should contain 3 mL methacholine. Six subjects should be tested and their order in session is 1-6. After the 6th person has been tested the 12.5 mg/mL solution is discarded, the nebuliser is cleaned and dried, and 3 mL of fresh 12.5 mg/mL solution is added. Six more subjects are tested and they are numbered 7-12. After the 12th person has been tested all solutions are discarded and the nebulisers are cleaned. The next session begins when new solutions are added. A session may be extended over one night only by placing the nebulisers containing solutions upright in the fridge, covered with parafilm.

THE MEFAR MB3 DOSIMETER

Quality control of Mefar dosimeter nebuliser output

The methacholine challenge protocol has been written assuming that each single inhalation delivers approximately 0.01 mL solution to the mouth.

All Mefar nebulisers in the study should be calibrated in Melbourne prior to use in the study.

Pressure control Mefar

The driving pressure of the Mefar dosimeter should be checked before the study starts and every four weeks thereafter. The method for checking and adjusting the driving pressure is available at:

[‘http://www.med.monash.edu.au/medicine/alfred/research/respiratory_medicine/mefar.html.’](http://www.med.monash.edu.au/medicine/alfred/research/respiratory_medicine/mefar.html)

Pressure control forms should be returned to the ECRHS II Co-ordinating Centre at completion of the study.

See Appendix E4 for Pressure Control sheet

Setting up the Mefar dosimeter

3 mL of methacholine solution should be placed in the appropriate nebuliser. A dry sterile mouthpiece should be connected for each new subject.

The Mefar should be set at:

- 1) inhalation time: 1 second
- 2) pause time: 6 seconds

The standard inhalation

The sequence of inhalation is:

- 1) Slow expiration to functional residual capacity
- 2) Place lips around mouthpiece to produce airtight seal
- 3) Slow inspiration to total lung capacity
- 4) Hold breath for at least three seconds

5) Remove mouthpiece and exhale

The procedure should be repeated after six seconds until sufficient inhalations for the dose have been performed. Inhalations may be performed on consecutive breaths if desired. Spirometric testing should be carried out two minutes after the dose. As soon as two FEV₁ manoeuvres have been recorded, the test should be continued with the next dose.

The end of the testing session

Solutions remaining in the nebulisers must be discarded and under no circumstances should they be returned to the storage containers. All nebulisers must be cleaned and dried. All mouthpieces must be cleaned, sterilised and thoroughly rinsed to ensure that there is no sterilising solution left on the surface.

THE METHACHOLINE PROTOCOL

Instructions for baseline spirometry

Perform full FVC manoeuvres as described previously for 'Baseline spirometry' (The forced expiratory manoeuvre). Record INITIAL FEV₁ and FVC. Calculate the BEST INITIAL FEV₁ as a percentage of the total predicted.

Measurement of control (post-diluent) FEV₁

The control FEV₁ is the FEV₁ measured following the inhalation of diluent. Four inhalations of diluent (WHITE nebuliser) are given, as described in 'The standard inhalation'.

Perform FEV₁ manoeuvres as described in 'Methacholine challenge' (The forced expiratory manoeuvre). Record CONTROL (POST-DILUENT) FEV₁. Calculate BEST CONTROL FEV₁ as a percentage of the BEST INITIAL FEV₁.

If the BEST CONTROL FEV₁ is less than 90% of the BEST INITIAL FEV₁ methacholine challenge is not carried out. Bronchoconstriction should be reversed by administering 400 µg salbutamol by MDI via a Volumatic and full FVC manoeuvres should be repeated.

If the BEST CONTROL FEV₁ is within 10% of the best initial FEV₁. Calculate 80% of the BEST CONTROL FEV₁. Calculate 90% of the BEST CONTROL FEV₁. Methacholine challenge may now be conducted following either the short or long protocol.

Dosing Schedule

In ECRHS I centres were able to decide whether to use one of two dosing schedules for methacholine challenge. In ECRHS II only one method should be used (Method 2 from ECRHS I protocol).

Choice of long or short protocol

Each subject can be challenged on the short or long protocol. The long protocol will increase by doubling doses and the short by quadrupling doses. Subjects most likely to react to methacholine should be tested on the long protocol. Subjects who are unexpectedly reactive and have been allocated to the short protocol may switch to the long protocol during the challenge to avoid severe bronchoconstriction. The choice of protocol for each subject will be assessed by the Main Questionnaire. The questions to be used to direct subjects to the long protocol may be decided locally, but the following are recommended:

Subjects who answered 'YES' to any one of Questions 1, 2, 3, 5, 11 or 14 in the Main Questionnaire, that is any subject who has:

- 1) had wheezing or whistling in their chest in the last 12 months (Q1)
- 2) woken with tightness of chest in the last 12 months (Q2)
- 3) had an attack of shortness of breath during the day while at rest in the last 12 months (Q3)
- 4) been woken by an attack of shortness of breath in the last 12 months (Q4)
- 5) trouble with their breathing (Q11)
- 6) ever had asthma (Q14)

Methacholine challenge protocol

CONC (mg/mL)	No of inhalations:		CUMULATIVE DOSE (mg)	DOSE LEVEL (As per ECRHS I)
	LONG	SHORT		
0.39	2		0.0078	3
0.39	2	4	0.0156	4
1.56	1		0.0312	5
1.56	2	3	0.0625	6
6.25	1		0.125	7
6.25	2	3	0.25	8

12.5	2		0.5	9
12.5	4	6	1.0	10
12.5	8	8	2.0	11

Changing from Long to Short protocol

If, during the short protocol, the FEV₁ falls 10% or more from the best control FEV₁, the subject should change protocol and receive the next dose level on the long protocol.

For example: A subject following the short protocol shows a fall of 10% after Dose 4 (four inhalations of

0.39 mg/mL). They should inhale Dose 5 (one inhalation of 1.56 mg/mL) next.

Short protocol:

Change to long protocol if FEV₁ falls below 90% of the BEST CONTROL FEV₁. Go to next dose level on long protocol.

STOP challenge if FEV₁ falls below 80% of the BEST CONTROL FEV₁

Long protocol:

STOP challenge if FEV₁ falls below 80% of the BEST CONTROL FEV₁

Completion of test

The methacholine challenge is complete when a cumulative dose 2 mg of methacholine has been reached.

It should be stopped sooner if:

- 1) there is greater than 10% fall in FEV₁ from the **BEST BASELINE FEV₁** following inhalation of diluent
- 2) there is greater than 20% fall in FEV₁ from the **BEST CONTROL FEV₁** following inhalation of any concentration of methacholine solution
- 3) the subject is not able to perform two technically satisfactory manoeuvres in five attempts following any dose level
- 4) the subject does not wish to carry on

Subjects may complain of mild chest tightness, coughing or wheezing but if lung function does not demonstrate a 20% fall in FEV₁ this is not an indication to stop the test

Reversal of bronchoconstriction

Four hundred micrograms of salbutamol will be given via a volumatic (see above for bronchodilator challenge). Perform full FVC manoeuvres as described in 'Methacholine challenge' 10 minutes after administration.

Record the POST-BRONCHODILATOR FEV₁ and FVC.

Calculate the BEST POST-BRONCHODILATOR FEV₁ as a PERCENTAGE of the BEST INITIAL FEV₁.

If the best post-bronchodilator FEV₁ is more than 90% of the best initial FEV₁ the test is over.

EACH CENTRE SHOULD PREPARE PROTOCOLS TO BE FOLLOWED IN THE EVENT OF A SUBJECT NOT RETURNING TO WITHIN 10% OF THE BASELINE.

BRONCHODILATOR CHALLENGE PROTOCOL

Four hundred micrograms of salbutamol should be administered by MDI as described in 'Bronchodilator challenge'. Perform full FVC manoeuvres as described in 'Baseline spirometry'. Record the POST-BRONCHODILATOR FEV₁ AND FVC.

THE INDOOR PROTOCOL

Specific objectives

1. To describe the distribution of exposure to house dust mite allergen in Europe.
2. To determine the relative and attributable risk of exposure to house dust mite allergen on the development of asthma-like symptoms, sensitisation to house dust mite, decline in lung function and change in bronchial reactivity in young adults.
3. To assess the levels of house dust mite allergen in the mattress of 200 young adults who have contributed health information as part of ECRHS II.

Methodology

THE SAMPLE

Each centre should recruit 200 people into this part of the study. Ideally subjects should come from the random sample, not have moved home and have provided blood for IgE assessment as well as questionnaire information. However in many centres high rates of migration among the sample may mean that other individuals need to be recruited.

To recruit the sample the following must be ascertained about each subject:

- Has moved house since the last survey
- Is random or symptomatic sample
- Has provided blood sample or not

Subjects should be recruited in the following order of priority to obtain a sample of 200 (it is assumed that everyone who has provided a blood sample has also completed the Main Questionnaire).

1. Not moved home, random sample, provided blood
2. Not moved home, symptomatic sample, provided blood
3. Not moved home random sample questionnaire only

4. Not moved home symptomatic sample questionnaire only
5. Been in current home for 5 years or longer, random sample, provided blood
6. Been in current home for 5 years or longer, symptomatic sample, provided blood
7. Been in current home for 5 years or longer random sample questionnaire only
8. Been in current home for 5 years or longer, symptomatic sample, questionnaire only
9. Anyone remaining from the random sample
10. Anyone remaining from the symptomatic sample

For some centres this means that home visits will only be conducted on random sample subjects who have not moved home and who have provided blood BUT for others the 200 home visits may include visits to subjects who have been in their homes for five years or longer and who have not provided a blood sample. It is important however that 200 samples are selected from each centre.

The homes of subjects who have conducted allergen avoidance should be visited but the action they have taken will be clearly documented using the indoor questionnaire.

Home visits must be spread over the period of one year to ensure that homes are visited in all seasons.

A brief questionnaire should be completed (*See Appendices F1 and F2 for Indoor Questionnaire and Instructions*). Some questions (shown on the questionnaire in bold) will be completed by the fieldworker without being read out but others require a response from the subject. These questions should be completed in the order given in the questionnaire.

House dust mite sampling should be conducted after completion of the questionnaire

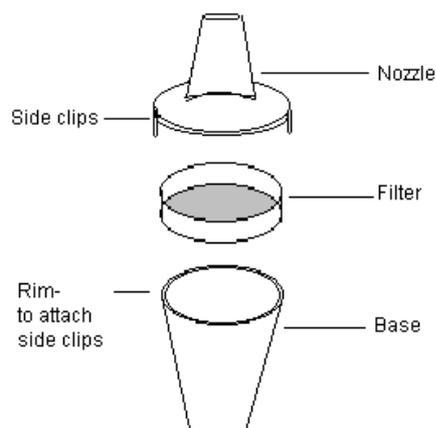
EQUIPMENT REQUIRED

- Vacuum cleaner (Electrolux Mondo 1150, 1300 watts)

- Dust sampling device, consisting of a ‘base’ and ‘nozzle’ (ALK-Abello, Horsholm, Denmark Cat no: 012 572) *You will need as many of these as number of visits per session (i.e. if you do three visits per day you will need three)*
- 200 Dust filters, consisting of a ‘filter’ and ‘lid’ (ALK-Abello, Horsholm, Denmark Cat No 012 570)
- Timer
- Template (0.8m x 1.25 m) to define area of mattress that will be vacuumed

METHOD

The bed in which the study subject is currently sleeping should be stripped of bed linen that is regularly changed. Any mattress covers/protectors that have been in place for at least 3 months should be left on the mattress.



The *filter unit* should be opened and the lid placed to one side.

The *base* should be attached to the vacuum cleaner hose and, while holding the vacuum upright, the *filter* positioned on the base. The *nozzle* should be secured firmly to the *base* over the *filter* by fitting the two side clips securely under the rim.

The 'one square metre' template (actually 0.8 x 1.25m²) should be placed on the bed over the area that the person sleeps (excluding the pillow area and excluding the feet area). The area within the template should be vacuumed for two minutes, vacuuming each 'quarter' of the area for 30 seconds using a 'zig-zag' motion to aid even coverage of the mattress surface. Throughout vacuuming the nozzle should be kept almost vertical to ensure good contact with the mattress.

When vacuuming is complete, before switching off the vacuum cleaner, the *nozzle* should be held upright for five seconds, so that the *sampling device* is pointing upwards and the dust can settle on the *filter*. The vacuum should then be switched off. The *nozzle* should be separated from the *filter* by carefully disengaging the side clips and the *lid* placed firmly on the filter. The *filter* unit should then be removed from the *base*.

The *filter* should be clearly marked with an identifying label given

Subject number

Centre number

Date sample taken

The closed *filter* should be then placed in the ziplock plastic bag provided.

After use, the nozzle should be washed with warm water and dilute detergent (washing up liquid) and left to dry. (The base does not need to be washed as it is not in direct contact with the dust.) A clean nozzle must be used for each dust sample taken.

Storage of Samples Prior to shipping to Central Laboratory

The cassette (in its plastic bag) should be placed in a freezer (-20°C) for 24 hours to kill house dust mites. This should be done within three days of taking the sample, e.g. if it is Friday and there is no access to a freezer, then freeze the sample on Monday.

Immediately after the cassette is taken out of the freezer, a 0.5g sachet of silica gel should be placed inside the plastic bag containing the cassette and the bag re-sealed. This will remove any condensation and prevent mould spores from germinating during storage. Cassettes should then be stored at room temperature, i.e. in a suitable laboratory

/ room at not more than 20°C, until the laboratory in London is ready to receive the samples.

The silica gel has an orange 'indicator dye' which turns white if saturated with moisture. Any sachets that are not orange to start with must not be used. During storage of dust cassettes the sachets should be checked monthly and any that have turned white should be replaced.

Transportation of samples

Samples should be sent at completion of the study (unless a request to send earlier is received from the central laboratory).

Samples should be sent by courier (not by mail) directly to the central laboratory at King's College London but no special arrangement needs to be made for the temperature for transportation. The laboratory must then be informed exactly when to expect deliveries of samples.

The house dust mite assays should be performed using a monoclonal antibody ELISA using reagents from Indoor Biotechnologies Ltd., and results made available to all centres on completion of analysis of the samples for all samples in a suitable format containing:

Subject number

Centre number

Date sample analysed

Der p 1 level in µg/g

Der f 1 level in µg/g

Results can be provided in µg/m² which are valid for between centre comparisons if identical vacuum cleaners and sampling times have been used. A quality control scheme should be operational and results provided to centres when all analyses for that centre are complete.

Training and quality control for home visits

A short video showing the dust mite sampling procedure (available from Co-ordinating Centre) should be shown to all fieldworkers during a locally arranged training meeting.

A locally identified supervisor will attend at least five home visits during the first ten home visits