

Final Report

PARSIFAL

**Prevention of Allergy
– Risk factors for Sensitisation In children
related to Farming and Anthroposophic
Lifestyle**

Contract number: QLK4-CT-1999-01391

**Period covered by the project:
February 1 2000 – January 31 2004**

Co-ordinator: Göran Pershagen

Contents

PARSIFAL Partners Information.....	5
1: Summary related to reporting period (1 Feb 2003 – 31 Jan 2004)	7
2: Executive summary related to reporting period	17
3: Description of workpackages	19
4: Technological Implementation Plan	38
5: Executive summary of the whole project.....	50
6: Detailed report related to overall project duration	53
6.1 Background	53
6.2 Objectives.....	53
6.3 Subjects and Methods.....	55
Subjects	55
Questionnaire	63
Clinical examination and blood sampling.....	65
Dust samples	69
Faecal sampling.....	74
Bronchial hyperresponsiveness (BHR) test.....	75
24-h-Diet Recall	77
Ethical permission	78
6.4 Scientific achievements.....	79
Background characteristics	79
Selected life style variables	88
Health outcomes and sensitisation	103
Dust sample analyses	117
Faecal analyses.....	124
Bronchial hyperresponsiveness (BHR) analyses.....	126
Validity and reproducibility of the food questionnaire	134
Farm analyses.....	143
Anthroposophic analyses.....	151
Dietary factors and their relation to asthma and allergy	163
6.5 Conclusions	168
6.6 Dissemination and exploitation of the results	170
6.7 Main literature produced	171
6.8 References:	172

Appendix 1 Country specific fieldwork documentations

Appendix 2 Country specific analyses

PARSIFAL Partners Information

Professor Göran Pershagen
Institute of Environmental Medicine
Karolinska Institute
Box 210
SE-171 77 STOCKHOLM
Sweden
Dir. tel: +46-8-524 874 60
Fax: +46-8-30 45 71
E-mail: Goran.Pershagen@imm.ki.se

Professor Charlotte Braun-Fahrländer
University of Basel
Institute for Social and Preventive
Medicine
Steinengraben 49
CH-4051 BASEL
Switzerland
Dir. tel: +41-61-270 22 20
Secretary: tel: +41-61-270 22 22
Fax: +41-61-270 22 25
E-mail: C.Braun@unibas.ch

Professor Bert Brunekreef
Institute for Risk Assessment Sciences
Utrecht University
PO Box 80176
NL-3508 TD, Utrecht
The Netherlands
For express deliveries: Yalelaan 2, 3584
CM, Utrecht NL
Office visiting address: Jenalaan 18a, 3584
CK, Utrecht NL
Dir. tel.: +31-30-2539490
Tel. Inst.: +31-30-2535400
Fax: +31-30-2535077
E-mail: b.brunekreef@iras.uu.nl

PD Dr Erika von Mutius
Dr von Haunersche Kinderklinik
Lindwurmstrasse 4
D-80337 MÜNCHEN
Germany
Dir. tel: +49-89-5160 2709
Fax: +49-89-5160 4452
E-mail: Erika.Von.Mutius@med.uni-muenchen.de

Professor Dr Josef Riedler
Children's Hospital
Department of Paediatric Pulmonology &
allergology
Müllner Hauptstrasse 48
A-5020 SALZBURG
Austria
Dir. tel: +43-662-4482 2619 (or 2601)
Fax: +43-662-4482 2604
E-mail: J.Riedler@lks.at

1: Summary related to reporting period (1 Feb 2003 – 31 Jan 2004)

Objectives

The overall aim of the project is to assess the role of certain environmental and lifestyle factors for the development of allergy in children. A novel approach is to focus primarily on factors offering protection in relation to allergy. Reduced exposure to such factors may contribute to explaining the rise in allergy in many western countries in recent decades. Identification of protective factors is probably crucial for successful prevention.

The project focuses on two groups of children with a low prevalence of atopic diseases and sensitisation, children of farmers and children with an anthroposophic life style. Initially, the project identified 15 000 children of farmers and in anthroposophic communities as well as controls in five European countries: Austria, Germany, the Netherlands, Sweden and Switzerland. Second, a questionnaire survey was carried out among the children and blood samples were obtained for determination of allergen specific IgE-levels. Third, measurements were performed of biological contaminants in stables and other indoor environments for some of the children. Fourth, the intestinal microflora was assessed for certain children based on fecal samples.

The objectives during the fourth year of study included finalisation of analyses of dust samples, preparation of data bases, data analyses and manuscript preparation.

Scientific/Technical progress

The project consists of six work packages (WPs). WP 1 includes five similar cross-sectional studies performed in Austria, Germany, Netherlands, Sweden and Switzerland, respectively. The studies use a uniform methodology developed within the ISAAC project, which is based on internationally validated questionnaires and analyses of blood samples. WP 2 is devoted to development of methods for assessment of health effects, primarily in relation to symptoms and serological markers associated with atopy. It also includes objective measurements of bronchial hyperreactivity. WP 3 focuses on exposure estimation using questionnaire data, with particular emphasis on dietary information which will be carefully validated.

WP 4 focuses on measurements of biological contaminants in different environments, primarily in stables and domestic environments. WP 5 includes analyses of fecal samples from children in the different groups, selected on the basis of dietary habits and other characteristics. In both WP 4 and WP 5 information on occurrence of atopy are used to select study subjects. WP 6 consists of an overall evaluation combining the results of WP 1-5.

The project structure and time table are described in Figures 1 and 2, as well as Tables 1 and 2.

During the fourth reporting period two meetings were held with participation from all partners, one in Utrecht 14-15 April 2003 and another in Basel 23-24 January 2004. Additional cleaning and corrections of the databases have been performed. The project brochure was finalized. Analyses of the association between anthroposophic life style features and childhood allergy have been performed and a manuscript was prepared. Some analyses of the fecal samples have been performed.

A manuscript was prepared on validation of dietary information in children. Preliminary analyses have been performed on the relation between dietary factors and allergy in children.

Analyses were performed of the data from the bronchial challenge tests and a manuscript based on the results was prepared.

The analyses of microbial agents in the dust samples were completed and a database containing measurement results was prepared. A manuscript was prepared on microbial agents in dust samples from the different exposure groups.

Analyses of the relation between farming characteristics and childhood allergy have been performed. A reporting of preliminary results was done at the project meeting in Basel.

A final report of the project was prepared.

Figure 1: Project structure

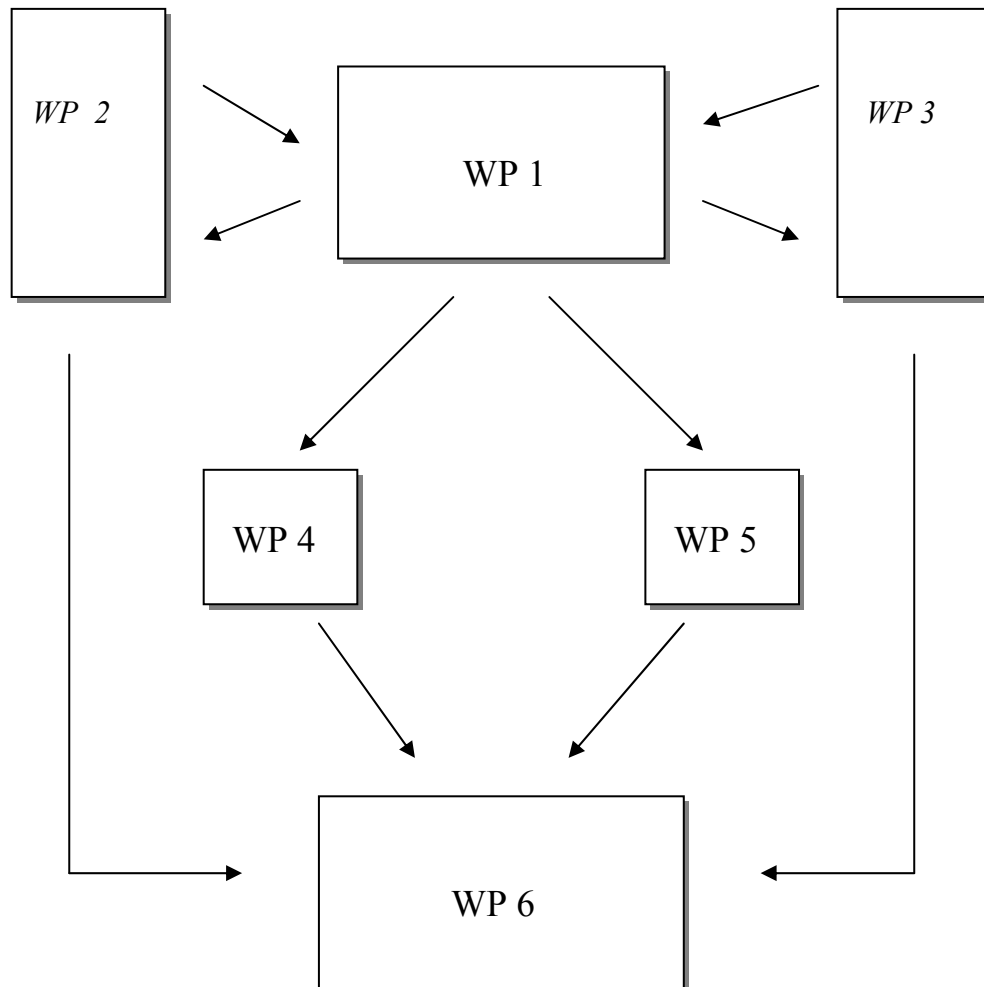


Table 1: Work package list

Work package No	Work package title	Responsible participant	Person-months	Start month	End month	Deliverable No
1	Epidemiological study design and data collection	P1	33	0	19	D1,D7
2	Assessment of health effects	P4	55	0	31	D4, D5, D11, D12, D17
3	Exposure assessment based on questionnaire	P2	28	0	25	D3, D8, D9
4	Dust collection methods and laboratory analysis of biological contaminants	P3	23	0	43	D2, D6, D10, D14, D15, D19, D21
5	Assessment of intestinal microflora	P1	20	13	43	D13, D16, D18, D22
6	Pooled analysis and overall assessment	P1	18	25	48	D20, D23, D24
Total			177			

Table 2: **Deliverables list**

Deliverable No	Deliverable title	Delivery month	Nature	Dissemination Level	Status
D 1	Protocol developed for selection of study subjects	3	O	RE	Complete
D 2	Protocol developed for dust collection	4	O	RE	Complete
D 3	Questionnaire developed for assessment of exposures	7	O	PU	Complete
D 4	Questionnaire developed for assessment of atopy	7	O	PU	Complete
D 5	Protocol developed for bronchial challenge tests	7	O	RE	Complete
D 6	Dust samples delivered to P3	13	O	CO	Complete
D 7	Databases created including results of questionnaire study in each centre	19	O	CO	Complete
D 8	Database created on exposure	25	O	CO	Complete
D 9	Report prepared on results of dietary validation	25	R	PU	Complete
D 10	Data base created including results of analyses of biological contaminants (Part A)	25	O	CO	Complete
D 11	Report prepared from bronchial challenge tests	25	R	PU	Complete
D 12	Blood samples delivered to Karolinska for IgE analysis	25	O	CO	Complete
D 13	Protocol developed for collection of fecal samples	25	O	RE	Complete
D 14	Report prepared based on analyses of biological contaminants (Part A)	31	R	PU	Complete
D 15	Delivery of dust samples for analysis of biological contaminants (Part B)	31	O	CO	Complete
D 16	Delivery of fecal samples to P1	31	O	CO	Complete

D 17	Data base created with results of blood analyses	31	O	CO	Complete
D 18	Data base created based on analyses of fecal samples	37	O	CO	Complete
D 19	Data base created based on analysis of biological contaminants (Part B)	37	O	CO	Complete
D 20	Creation of data base including data from questionnaires and blood samples in all centres	37	O	CO	Complete
D 21	Report prepared based on analyses of biological contaminants (Part B)	43	R	PU	Complete
D 22	Report prepared based on analyses of fecal samples	43	R	PU	Complete
D 23	Reports prepared on protective factors for allergy in children	46	R	PU	Complete
D 24	Overall evaluation report	48	R	PU	Complete

2: Executive summary related to reporting period

Contract no QLK4-CT-1999-01391

Reporting period: 1 Feb 2003 – 31 Jan 2004

Title: PARSIFAL

Objectives: The PARSIFAL project focuses on farm children and children with an anthroposophic lifestyle, because some earlier studies have shown a lower prevalence of atopic diseases and sensitisation in these groups. The study was performed to assess if there are any differences in the possible protecting effects from these lifestyles in five different countries in Europe (Austria, Germany, Holland, Sweden and Switzerland). A further aim has been to assess the role of certain environmental and lifestyle factors for development of allergy in children.

Scientific achievements: The study included children, aged 5-13, from farm families, children with an anthroposophic lifestyle and corresponding reference groups in Austria, Germany, Holland, Sweden and Switzerland. The final study population consisted of 14893 children, 19% farm children, 37% farm references, 31% Steiner school children and 14% Steiner references. A questionnaire was distributed to the parents to obtain information on environmental exposures, life style, socioeconomic conditions, history of infections, diet, contact with animals, and on symptoms of bronchial asthma, allergic rhinoconjunctivitis, food allergy/urticaria and atopic eczema. One third of the children left blood samples for measurements of allergen-specific IgE against common inhalant and food allergens. Furthermore, dust samples were examined regarding certain microbial contaminants and allergens in the farm and anthroposophic environment. To assess the microflora of the gut in relation to certain exposure characteristics, faecal samples from about 400 children were collected.

Main deliverables: There is a lower prevalence of both subjective and objective markers of atopic disease when comparing the farm children and their reference group as well as when comparing the Steiner school group and their reference group.

The protective effect of farming is found for all of the studied atopy outcomes, both self-reported, as for example current hay fever symptoms, wheezing, atopic eczema and asthma, and objective in the form of sensitisation (serum-IgE >0.35 kU/l) when studying all countries together. The risk of having current hay fever or being sensitised is only about 50% for the farm children compared to their references. In the German subset the results look quite the same as for all the children, whereas the protective effect among farm children in the other countries appears consistent mostly for hay fever and sensitisation.

The protective effect of the anthroposophic life style is found for all of the studied outcomes except for current wheezing. The risk of having current hay fever or being sensitised is about 75% for the Steiner school children compared to their references. Also here there were some differences between the countries. Hardly any effect from the anthroposophic life style is found in the Austrian subsample, and in Switzerland a clear lower risk in the Steiner group was seen only for sensitisation. Consistent protective effect of the anthroposophic life style is found primarily in Germany, the Netherlands and Sweden.

The first results from the house dust analyses demonstrate that farm children and - to a lesser extent - Steiner children, are exposed to higher levels of endotoxin, EPS and glucans, than their respective references. This may contribute to the lower prevalence of atopy. Further analyses on the relationship between components in the dust samples and atopy and asthma are in progress.

We found no substantial differences in the association between bronchial hyperresponsiveness (BHR) test to hypertonic saline and questionnaire information on 'asthma' and 'wheeze' among the farm children, Steiner school children, farm reference children and Steiner reference children.

This suggests that the comparisons of symptom rates between the different groups in our study are valid.

Preliminary analyses of the faecal samples suggest differences in the intestinal bacterial flora between Steiner children and their references, as well as a presence of certain bacteria in children from pig farms.

In view of the limited information available on the quality of dietary information for children obtained via questionnaire we investigated validity and reproducibility of the dietary data. Overall we found moderate to acceptable validity and moderate to substantial reproducibility in the parental reports of their children's diet. The quality was considered sufficiently accurate for diet-disease analyses of most foods.

Socio-economic relevance and conclusions: The study confirms that there is a protective effect from a farming and anthroposophic lifestyle for the development of allergy in children. Extensive ongoing analyses will further clarify the role of specific environmental and life style factors for this effect. When these results become available they may be used as a basis for preventive measures.

Keywords: Allergy, anthroposophic lifestyle, asthma, children, diet, environmental factors, farming lifestyle, prevention, sensitisation.

3: Description of workpackages

WP 1: Epidemiological study design and data collection via questionnaire

Starting date: 0

Completion date: 19

Current status: Completed

Responsible partner: P1

Person-months per partner and total: P1 (9), P2 (6), P3 (6), P4 (6), P5 (6), Total (33)

Objectives

The WP aims to select study subjects and carry out epidemiological studies on preventive factors for development of allergy among children in farming and anthroposophic communities in five European countries. The focus is on different types of animal contact, various infections and certain dietary habits. A further aim is to serve as a sampling frame for selection of study subjects to be investigated more thoroughly in relation to biological contaminants (WP 4) and microflora of the gut (WP 5).

Methodology and study material

The study is based in Austria, Germany, The Netherlands, Sweden and Switzerland. In each country a total of around 1200 children aged 6 to 12 years will be included among farmers, families with an anthroposophic life style and a control group. Local contacts have recently been established in each of the countries with farming communities in relation to earlier epidemiological studies. In one of the countries a study has already been performed in anthroposophic communities and such contacts have also been developed in the other countries. The studies will include a variety of farming environments from those with heavy reliance on livestock to those based on grain production.

Study subjects will be included based on a selection of certain schools. Farming children are identified from registries, Farmer Organizations or school classes in relevant areas. For anthroposophic children the selection is based on attendance in Steiner schools. School classes without farming or anthroposophic children are used as controls. Schools in cities or highly urbanised areas will be avoided to enhance comparability between the groups.

Information on environmental and life style factors as well as on clinical symptoms and biological markers of atopy will be obtained according to the ISAAC II protocol, with some additions based on WP 2 and WP 3. Briefly, this includes a questionnaire which has been thoroughly validated and tested, including translation and backtranslation, and blood sampling for IgE determination. The classification of atopy based on the different sources of data will also follow the ISAAC protocol. Successful implementation of a similar methodology has been demonstrated by all the participating centers.

Progress during the first reporting period:

During the first reporting period a protocol for subject selection has been produced as planned, and all centres have adapted these common instructions to local needs to accomplish subject selection. Ethical approvals have been obtained in all centres. The questionnaire distribution and collection period started in month 9 and is expected in the last centre for the final subjects to be finalised by mo 16, although the bulk of all questionnaires were collected by the end of mo 12, as planned. All centres expect to be able to supply a database of questionnaire data to P1 after 18 months, as planned. All centres will meet or exceed the planned recruitment numbers.

Progress during the second reporting period:

During the second reporting period the questionnaire distribution and collection has been finalised in all centres except the Netherlands where there has been delay in collecting data from farmers' children and their controls due to the outbreak of Foot-and-Mouth disease in 2001. Thus, all centres but the Netherlands have supplied a database of questionnaire data to P1 and the Netherlands will supply a sample database with available data by month 25. All centres have met or exceeded the planned recruitment numbers.

Progress during the third reporting period:

During the third reporting period the Netherlands finalised the questionnaire distribution and collection from farmers' children and their controls, and supplied a database of questionnaire data to P1. Thus, all centres have supplied a database of questionnaire data to P1. From these data, a database containing information from the questionnaires from all centres has been produced and the data cleaning and corrections have been completed.

Deliverables

WP 1 will produce a protocol [**D1, DONE**] to generate a sample of at least 1 200 school children aged 6-12 years in each centre from farming and anthroposophic communities. From this sample a database will be produced by each centre according to a standardised format including close to 1 000 children [**D7, DONE**]. The data base will contain information from the questionnaire focusing on environmental exposures, life style, socioeconomic conditions, history of infections, diet and, contact with animals, as well as on symptoms of bronchial asthma, allergic rhinoconjunctivitis, food allergy/urticaria and atopic dermatitis.

Milestones

A protocol for selection of study subjects will be available by mo 3 [**D1, DONE**]. Selection of study subjects will be finalised during the first 6 mo of the project [**DONE**]. Data collection via questionnaire will continue until mo 12 [**DONE**]. It is expected that the data base within each centre will be created by mo 18 [**D7, DONE**].

WP 2: Assessment of health effects

Starting date: 0

Completion date: 31

Current status: Completed

Responsible partner: P4 (& P5 & P1 lab)

Person-months per partner: P1 (18), P2 (10), P3 (9), P4 (9), P5 (9), Total (55)

Objectives

The objective of this work package is the assessment of asthma, hay fever, atopic dermatitis and allergic sensitisation in children of farming and anthroposophic families as well as in controls.

Methodology and study material

To assess prevalence of atopic diseases, the questionnaire distributed to the parents also includes questions on symptoms and diagnosis of asthma, hay fever and atopic dermatitis according to the internationally standardised ISAAC protocol. The ISAAC questions on asthma have been validated with respect to bronchial hyperresponsiveness, and translated into the different languages of the participating centres.

In WP2 parents are asked for their written informed consent that their children participate in venous blood sampling. Approximately, 10 ml will be drawn for measurement of specific IgEs to common inhalant and food allergens (RAST CAP tests, Pharmacia and Upjohn, Sweden) according to the ISAAC phase II protocol. Sx1 (inhalative allergens) and fx5 (nutritional allergens) screening tests are done first. In case of positivity, the following allergens are tested separately: Dermatophagoides pteronyssinus, Lepidoglyphus destructor, grass pollen, tree pollen, cat and horse epithelium. Since all sera will be stored, there is later the opportunity to analyse specific IgE against cow among farmers' children and dog and Dermatophagoides farinae among those children whose house dust will be analysed for the content of Can f 1 and Der f 1. All RAST analyses will be performed centrally at the Department of Clinical Immunology at the Karolinska Hospital, Stockholm, Sweden.

A subsample of about 1 000 children, including all with wheeze and a random sample of those without wheeze will undergo bronchial challenge with 4.5% hypertonic saline inhalation. A minimum of two baseline spiromograms will be recorded and the highest of two reproducible (within 5%) measures of FEV1 will be recorded as baseline FEV1. Bronchial reactivity will be assessed by changes in FEV1 after inhalation of nebulised saline using ultrasound nebulisers (DeVilbiss Sunrise Medical, Langen, Germany). All children will be asked to withhold all asthma medications for at least 12 hours. In children with a baseline FEV1 of less than 75% predicted, no bronchial challenge will be performed and an inhaled bronchodilator (salbutamol) will be administered.

Progress during the first reporting period:

A protocol for assessment of health effects has been finalised as planned, consisting of three components – questionnaire assessment, blood sampling and bronchial challenges. Questionnaire assessment is underway since month 9, nearing completion in all centres. Blood sampling has been started in one centre in month 13, followed by the other centres. A practice session has been held for 3 centres which are about to start performing bronchial challenges and a similar session is planned for 2 centres scheduling a somewhat later start for bronchial challenges. All health effect assessments in all centres will be finalised by mo 24 as planned. Blood samples will be delivered to the lab (P1) during mo 13-16 (local P1 study), 17-18 (3 centres) and 22 (1 centre) and will thus be delivered according to plan before mo 24 and analysed well in advance of the planned deadline of mo 30.

Progress during the second reporting period:

Based on the protocol for assessment of health effects finalised early in the project, consisting of three components – questionnaire assessment, blood sampling and bronchial challenges, most of the data collection is now completed. Questionnaire assessment is completed in all centres but the Netherlands. Blood sampling and bronchial challenges likewise. Blood samples from all centres but the Netherlands were delivered to the lab (P1) and analysed within the second reporting period. Information on the results is being sent out to participants.

Progress during the third reporting period:

Assessments of health effects – questionnaire assessment, blood sampling and bronchial challenges – have been finalised for all centres. Blood samples from all centres have been delivered to the lab (P1), analysed, and the results have been sent out to the parents. A database containing results from the blood analyses from all centres has been prepared. Likewise, a database containing results from bronchial challenges has been created.

Deliverables

This work package will deliver the prevalence of asthma, hay fever, atopic dermatitis, atopic sensitisation and airway hyperresponsiveness to be used as health outcome measures for further data analysis.

WP2 will generate a database on roughly 3 600 children containing information on health parameters from questionnaires and blood samples as well as bronchial challenge data for 1000 children. Each partner will be responsible for data entry of the population studied locally.

Milestones

A protocol for assessment of health effects will be finalised by mo 6 [**D4, D5 - DONE**]. Blood will be sampled and bronchial challenges will be performed from mo 7 to mo 24 [**D11, D12 - DONE**]. Blood samples will be analysed centrally by mo 30 [**D17 - DONE**]. We expect that entry of all questionnaire, pulmonary function data and blood test data will be finalised by mo 36 [**D20 of WP6 - DONE**].

WP 3. Exposure assessment based on questionnaire

Starting date: 0

Completion date: 25

Current status: Completed

Responsible partner: P2

Person-months per partner and total: P1 (2), P2 (20), P3 (2), P4 (2), P5 (2), Total (28)

Objectives

The WP aims to develop an instrument for collection of information on risk factors and potential protective factors in relation to allergies based on a carefully validated and tested questionnaire (including translation and backtranslation). Special emphasis will be given to the assessment of different dietary habits associated with farming or an anthroposophic lifestyle.

Methodology and study material

Information on exposures associated with the home environment (bedroom sharing, pets, smoking, cooking and heating fuel, air conditioning, damp spots and visible moulds, floor covering, insulation), the family environment (number of younger and older siblings, family history of diseases, child's personal history of infections and immunisation, birthweight, breastfeeding) and socio-economic conditions will be collected by questionnaire based on the internationally validated and translated ISAAC phase II questions. Exposures and lifestyle factors related to living on a farm (contact to farm animals, time spent in barns and with agricultural activities) will be assessed based on the questionnaires developed for recent studies in Switzerland, Germany and Austria. Information on factors associated with anthroposophic lifestyle will be collected based on the questions developed for a recent Swedish study. The questions available in German and Swedish will be translated and backtranslated into the other two languages.

Long-term diet will be assessed using a semiquantitative food frequency questionnaire (FFQ). The FFQ will be designed to specifically include food items that are discriminating between our study population (children of farming and anthroposophic families) and the general population. The mother of the child included in the study will be asked to provide the relevant dietary information on the child. The FFQ will be validated in a subsample of representative families with a similar food frequency questionnaire administered by telephone.

Progress during the first reporting period:

A questionnaire for exposure assessment was ready as planned and was ready for distribution and printed in the first centre by mo 9. Questionnaire assessment is underway since mo 9, nearing completion in all centres. Dietary validation has been started in mo 13 and is expected to be completed by mo 18 as planned.

Progress during the second reporting period:

The food frequency questionnaire has been administered in all centers with some delay in the Netherlands. The dietary validation will continue until months 32, because it has to be based on the cleaned dataset including the questionnaires of all centers

Progress during the third reporting period:

During the third reporting period the distribution of the food frequency questionnaire was completed in all centres. A database containing the results from the food frequency questionnaire and the dietary validation has been created. The work with the report on results from the dietary validation is ongoing.

Progress during the fourth reporting period:

A report on the dietary validation has been produced.

Deliverables:

WP3 will generate a database containing information on environmental exposures, lifestyle, socio-economic conditions, history of infections and immunisation, contact to animals and dietary habits of a total of close to 5000 children aged 6-12 years, from farming, anthroposophic and control families based on the data collection within WP1 **[D8, DONE]**. A report will be produced on the validation studies regarding dietary information in the questionnaires **[D9, DONE]**.

Milestones

The questionnaire for assessment of exposure will be developed by mo 6 **[D3, DONE]**. The dietary validation continues until mo 18 and a report based on the validation study is expected by mo 24 **[D9, DONE]**. The overall exposure database containing information from each centre is expected at the same time **[D8, DONE]**.

WP 4: Dust collection methods and laboratory analysis of biological contaminants

Starting date: 0

Completion date: 43

Current status: Completed

Responsible partner: P3 (& P1 some lab analyses)

Person-months per partner and total: P1 (2), P2 (6), P3 (11), P4 (2), P5 (2), Total (23)

Objectives

The objective of this work package is to prepare and organise the dust collection (to be carried out in the participating countries) and to analyse the dust samples for microbial contaminants and allergens in a central laboratory.

Methodology and study material

All 6000 participants will be sent dust collection bags for use in their own vacuum cleaners, to collect samples from living room floors and child mattresses using a technique developed and field-tested by the coordinating centre. Assuming a response of about 2/3 to this part, we anticipate to collect dust samples from 4000 homes. Of these, we will analyze 1000, contrasting homes of children with allergic asthma to homes of control children (part A).

Furthermore, in a random sample of 250 farms, 125 homes of anthroposophic children, and 125 control homes, we will study biological contaminants in detail (part B). We will collect dust during home visits with vacuum cleaners equipped with ALK filter holders. Glass fibre filters with known low background of the bacterial and mould contaminants will be used for those. Dust samples will be taken from 1 m² of carpets or rugs for 2 minutes, or from 2 m² of smooth floors (when no carpets or rugs are present). Mattress surfaces will be sampled entirely for 2 minutes without removing covers/underblankets and sheets. In farm stables or barns, surface samples will be taken in similar fashion as floor samples.

For measurement of bacterial endotoxin we use the Limulus Amebocyte Lysate (LAL) assay. For measurement of mould-derived extracellular polysaccharides (EPS) in dust extracts we use a sandwich Enzyme Immuno Assay (EIA) that was developed to quantitate with high sensitivity EPS from *Penicillium* and *Aspergillus* species, which may predominate in many indoor environments. For measurement of $\beta(1\rightarrow3)$ -glucans we use an assay with rabbit antiserum against $\beta(1\rightarrow3)$ -glucans, using a $\beta(1\rightarrow3)$ -glucan-protein conjugate.

Common allergens are measured with assays provided by Indoor Biotechnologies⁸ based on monoclonal antibodies for house dust mite major allergens *Der p1* and *Der f1*, and cat (*Fel d1*) and dog (*Can f1*) major allergens. Storage mite allergens will be measured by P1 with a specific EIA developed and used in that laboratory. Allergens from cow dander and pig urine will be measured with IgG₄ inhibition Enzyme Immune Assays.

Progress during the first reporting period:

Standard operating procedures and field protocols have been developed for Part A (sampling by parents) and Part B (sampling in homes and stables by fieldworkers). Sampling material for Part A have been distributed to the centres and Part A sampling has been started in several centres and will be finalised in all centres by mo 18 and sent to P3 lab. This represents a delay of about 6 months according to plan but will not affect the delivery of analysis results from the lab for Part A. Part B planning is according to plan.

Progress during the second reporting period:

Sampling sets for part B (stable dust and dust taken by fieldworkers) have been distributed to all centres. Stable dust and house dust samples taken by field workers of some centres (part B) were received by P3 and are currently being extracted and analysed. With the exception of the Dutch centre all sock dust samples (part A) have been collected and have been or will be shipped soon to the centre in the Netherlands for extraction and analyses.

Progress during the third reporting period:

All samples (part A and part B) have been sent to the centre in the Netherlands (P3) for extraction and analyses. All samples (part A and part B) have been extracted. Analyses of allergens and endotoxin have been completed, while the analyses of EPS and glucans are ongoing. A database containing results of analyses of biological contaminants has been created. The work with the report based on analyses of biological contaminants is ongoing.

Progress during the fourth reporting period:

Analyses of EPS and glucans in dust samples have been completed. A report on biological contaminants in dust samples has been prepared.

Deliverables

This work package will deliver the concentrations of allergens, endotoxin, EPS and glucans in dust to be used in further data analysis as well as reports based on the results of the measurements.

Milestones

The first milestone is the distribution of filter materials and standard operating procedures for dust collection to the centres. This will be after 3 months into the project. Included in this milestone is also the completion of standard operating procedures for extraction and analysis [D2, DONE]. The second milestone is the return of the samples to the partner 3 laboratory for extraction and analysis. This will be after one year into the project although it is foreseen that samples will be delivered to the lab in batches. This concerns Part A, sampling by parents, deliverable D6. [D6, DONE] Delivery of samples from part Part B, sampling by fieldworkers, is foreseen after mo 30, as was also initially planned, deliverable D15. [D15, DONE]. The third milestone will be the completion of the laboratory analysis of the samples including the production of the database. [D10, DONE]. The fourth milestone is the report of part A, which will be finalised after 2.5 years [D14, DONE]. The database for Part B is ready [D19, DONE] The report of part B is delivered [D21, DONE].

WP 5: Assessment of intestinal microflora

Starting date: 13

Completion date: 43

Current status: DONE

Responsible partner: P1

Person-months per partner and total: P1 (12), P2 (2), P3 (2), P4 (2), P5 (2), Total (20)

Objectives

This WP has an aim to assess the microflora of the gut in relation to certain exposure characteristics, such as use of antibiotics and history of infections as well as dietary habits, including consumption of unpasteurised milk and vegetables preserved by fermentation with lactobacilli. Furthermore, the intestinal microflora will also be related to the occurrence of atopy. WP 1 will generate the sampling frame for selection of study subjects according to these criteria, thus enabling a focus on "extreme" groups in relation to exposure and disease outcome to enhance efficiency. The microflora of the gut will be assessed using a variety of biological markers based on analyses of fecal samples.

Progress during fourth reporting period:

Microbial analysis of the dust samples has been performed: A report based on these results has been prepared.

Methodology and study material

Fecal samples will be obtained from a total of 400 children based on the information obtained in the questionnaire. The sample includes 50 children each in "high and low" categories related to consumption of unpasteurised dairy products, use of antibiotics, contact with livestock, and consumption of fermented vegetables. Furthermore, about half of the children should be atopic. The fecal samples will be obtained according to a protocol developed for an ongoing study of infants in an anthroposophic community. Analyses will include determination of a variety of bacteria based on culture, including brucella, Clostridium difficile, coliforms, enterococci and lactobacilli as well as genotyping.

Progress during the first reporting period:

According to plan, work is ongoing on the details of a protocol for collection of fecal samples to be ready by the end of mo 19, somewhat in advance of planned mo 24.

Progress during the second reporting period:

A protocol for collection of fecal samples was developed and fecal sampling is now ready in some centres and underway in others.

Progress during the third reporting period:

During the third reporting period the fecal sampling was completed in all centres, and all samples were delivered to P1. A database with information about the fecal samples from all centres was created. The microbiological analyses have been initiated.

Progress during the fourth reporting period:

Microbiological analyses of the fecal samples were performed using DNA-based techniques. A preliminary report including results from the fecal analyses was prepared.

Deliverables

The WP will generate a list of children from the five participating countries with certain features relating to exposure and atopy. Following analysis of the fecal samples the data base will also include information on growth of specific bacteria and certain strains based on biochemical fingerprinting. A report will focus on associations between fecal markers of the intestinal microflora and characteristics of exposure as well as atopy.

Milestones

The selection of study subject will start as soon as relevant information from WPs 1 to 3 become available, which is expected by mo 24 from the commencement of the whole project, **[D13, DONE]**. Fecal sampling is expected to take 6 mo **[D16, DONE]** and analysis of the samples continues until mo 36 of the project **[D18, DONE]**. The final report will be available by mo 42 **[D22, DONE]**.

WP 6: Pooled analyses and overall assessment

Starting date: 25

Completion date: 48

Current status: Completed

Responsible partner: P1

Person-months per partner and total: P1 (6), P2 (3), P3 (3), P4 (3), P5 (3), Total (18)

Objectives

This WP will use the databases generated by WPs 1 to 3 for a pooled analysis aimed at identifying protective factors for development of atopy in children. Combined analysis of the data in the different countries will greatly enhance the precision of risk estimates. Furthermore, information will be used from WPs 4 and 5 to assess the influence by exposure to biological contaminants and the microflora of the gut.

Methodology and study material

Pooled analyses will be performed based on the information obtained in the studies from Austria, Germany, the Netherlands, Sweden and Switzerland (WP 1 to 3). It is expected that this will include a total of about 5000 children from farming and anthroposophic communities as well as among controls. Data collection and creation of databases uses a uniform methodology, which facilitates combined analysis. The focus in the analyses will be on comparisons of the occurrence of allergy in the different study groups as well as on associations between specific exposures and atopy. Factors receiving particular interest include dietary habits, history of infections and contact with animals. Information on a number of other risk factors will also be available, such as heredity, passive smoking, breastfeeding, number of siblings etc, which will enable assessments of interactions and confounding control. The large database in the combined analysis facilitates detailed investigations of subgroups, such as assessments of interactions.

Based on the results of the pooled analysis, as well as from WP 4 and WP 5 an overall evaluation will be performed of the role of certain risk factors for development of allergy in children, with particular emphasis on protective exposures. It is expected that information from the measurements of biological contaminants and determination of the intestinal microflora could be of great interest in understanding mechanisms behind development of allergy. Overall, the results will be used to indicate useful directions for prevention of allergy.

Progress during the first reporting period:

A draft common data format and variable list has been drawn up and discussions regarding data cleaning and corrections has been initiated. Initial delivery of questionnaire data from all centres to P1 foreseen by mo 18

Progress during the second reporting period:

A common data format and variable list has been drawn up and data cleaning and corrections are ongoing at the coordinating centre. All centres but the Netherlands have supplied a database of questionnaire data to P1 and the Netherlands will supply a sample database with available data by month 25. Some descriptive exploratory analyses have started.

Progress during the third reporting period:

A common database structure has been drawn up. The data cleaning and corrections have been completed at the coordinating centre. The responsibility for analysis and preparation of reports regarding different parts of the project has been distributed among the partners and some analyses have started.

Progress during the fourth reporting period:

Pooled analyses of the data has been performed and a final report was prepared.

Deliverables

A common database will be created based on the information obtained in WPs 1 to 3. The responsibility for analysis and preparation of reports regarding different parts of the project will be distributed among the partners. Results of combined analyses and from WP 4 and 5 are used to produce a report focusing on strategies for allergy prevention.

Milestones

The database for the pooled analyses will be set up by mo 36 after the start of the whole project **[D20, DONE]**
Data analyses will continue for almost a year **[D23, DONE]**. The final report based on the evidence generated in WP 1-6 is available by mo 48 **[D24, DONE]**

ROLE OF PARTICIPANTS

Partner 1: Institute of Environmental Medicine and Department of Medicine at the Karolinska Hospital, Karolinska Institutet, Stockholm, Sweden

Principal investigator: (project co-ordinator)
Scientific staff:

Göran Pershagen, MD, PhD
Tobias Alfvén, MD, PhD
Johan Alm, MD, PhD
Anna Bergström, PhD
Helen Flöistrup, MSC
Marianne van Hage-Hamsten, MD PhD
Fredrik Nyberg, MD, PhD, MPH
Annika Scheynius, MD, PhD
Jackie Swartz, MD
Magnus Wickman, MD, PhD

Objectives

P1 will co-ordinate the whole project and is responsible for WPs 1, 5 and 6. These WPs involve epidemiological study design and questionnaire based data collection among farming and anthroposophic children in five European countries (WP1), assessment of the intestinal microflora in relation to certain dietary and environmental factors associated with atopy (WP5), and overall evaluation of the role of certain protective factors for childhood allergy (WP6). P1 is also primarily responsible for the IgE analyses in the blood samples (WP2) and for analysis of storage mites in the dust samples (WP4).

Deliverables

- | | |
|------|--|
| WP1: | 1) Protocol for selection of study subjects (D1)
2) Database including results from questionnaire study in each centre (D7) |
| WP2: | 1) Delivery of blood samples to P1 lab finalised (D12)
2) Data base created based on analyses of blood samples (D17) |
| WP3: | 1) Delivery of database on exposure to P1 (D8)
2) Delivery of database on dietary validation to P2 (D9) |
| WP4: | 1) Dust samples delivered to P3 (part A) (D6)
2) Data base created including results of analyses of biological contaminants, Part A (D10)
3) Delivery of dust samples to P3 for analysis of biological contaminants, Part B (D15)
4) Data base created based on analyses of biological contaminants, Part B (D19) |
| WP5: | 1) Protocol for collection of fecal samples (D13)
2) Delivery of fecal samples to P1 lab (D16)
3) Data base created based on analyses of fecal samples (D18)
4) Report prepared based on analyses of fecal samples (D22) |
| WP6: | 1) Database including data from questionnaires and blood samples in all centres (D20)
2) Reports prepared on protective factors for allergy in children (D23)
3) Overall evaluation report (D24) |

Research activities during the fourth reporting period:

During the fourth reporting period we have continued to coordinate the project. Two meetings were held with participation from all partners, one in Utrecht 14-15 April 2003 and another in Basel 23-24 January 2004. Additional cleaning and corrections of the database have been performed. The project brochure was finalized. Analyses of the association between anthroposophic life style features and childhood allergy have been performed and a manuscript was prepared. Some analyses of the fecal samples have been performed a final report of the project was prepared.

Significant difficulties and delays experienced during the fourth reporting period:

The final report was prepared with a delay of about one month because of late incoming material, but cost statements were submitted in time. The fecal analyses have been more complex than anticipated because a new DNA-based methodology was introduced.

Partner 2: University of Basel, Switzerland

Principal investigator: Charlotte Braun-Fahrlander
 Scientific staff: Karin Michels, PhD
 Marco Waser
 Roger Lauener
 Felix Sennhauser

Objectives

The objectives were to develop a comprehensive questionnaire including questions on dietary habits, to select the study subjects, to distribute questionnaires, and to start blood and dust sampling.

Deliverables

- WP1: 1) Protocol for selection of study subjects, local section (D1)
 2) Delivery of database including results from questionnaire study to P1 (D7)
- WP2: 1) Delivery of blood samples to P1 lab (D12)
- WP3: 1) Questionnaire developed for assessment of exposures (D3)
 2) Delivery of database on exposure to P1 (D8)
 3) Delivery of database on dietary validation to P2(D9)
- WP4: 1) Dust samples delivered to P3 (part A) (D6)
 2) Delivery of dust samples to P3 for analysis of biological contaminants, Part B (D15)
- WP5: 1) Delivery of fecal samples to P1 lab (D16)
- WP6: 1) Database including data from questionnaires and blood samples in all centres (D20)
 2) Reports prepared on protective factors for allergy in children (D23)
 3) Overall evaluation report (D24)

Research activities during the fourth reporting period:

A manuscript was prepared on validation of dietary information in children. The partner hosted the project meeting in Basel 23-24 January 2004. Preliminary analyses have been performed on the relation between dietary factors and allergy in children.

Partner 3. Institute for Risk Assessment Sciences (IRAS), Division of Environmental and Occupational Health. Utrecht University, The Netherlands

Principal Investigator: Bert Brunekreef, PhD
 Scientific staff: Gert Doekes, PhD
 Dieneke Schram, MSc

Objectives

The role of P3 is the co-ordination of the dust collection and associated laboratory work. In addition, this partner will conduct the Dutch field study, which involves participation in WP 1 to 5, and contribute to analysis and reporting of the pooled material (WP6).

Deliverables

- WP1: 1) Protocol for selection of study subjects, local section (D1)
 2) Delivery of database including results from questionnaire study to P1 (D7)
- WP2: 1) Delivery of blood samples to P1 lab (D12)
- WP3: 1) Delivery of database on exposure to P1 (D8)
 2) Delivery of database on dietary validation to P2 (D9)
- WP4: 1) Protocol developed for dust collection (Part A and B) (D2)
 2) Dust samples delivered to P3 (part A) (D6)
 3) Data base created incl. results of analyses of biological contaminants, Part A (D10)
 4) Report prepared based on analyses of biological contaminants, Part A (D14)
 5) Delivery of dust samples to P3 for analysis of biological contaminants, Part B (D15)
 6) Data base created based on analyses of biological contaminants, Part B (D19)
 7) Report prepared based on analyses of biological contaminants, Part B (D21)
- WP5: 1) Delivery of fecal samples to P1 lab (D16)
- WP6: 1) Database including data from questionnaires and blood samples in all centres (D20)
 2) Reports prepared on protective factors for allergy in children (D23)
 3) Overall evaluation report (D24)

Research activities during the fourth reporting period:

The analyses of microbial agents in the dust samples were completed and a database containing measurement results was prepared. The partner hosted the project meeting in Utrecht 14-15 April 2003. A manuscript was prepared on microbial agents in dust samples from the different exposure groups.

Partner 4: Ludwig Maximilian Universität Munich, Germany

Principal investigator: Erika von Mutius, MD MSc
 Scientific staff: Marcus Benz, MD
 Jörg Budde, MD
 Rob van Strien, PhD

Objectives

P4 together with P5 is responsible for design and implementation of WP2. Furthermore, this partner will contribute to the overall analysis and reporting, particularly regarding farming characteristics and childhood allergy.

Deliverables

- WP1:
 - 1) Protocol for selection of study subjects, local section (D1)
 - 2) Delivery of database including results from questionnaire study to P1 (D7)
- WP2:
 - 1) Questionnaire developed for assessment of atopy (D4)
 - 2) Delivery of blood samples to P1 lab (D12)
- WP3
 - 1) Delivery of database on exposure to P1 (D8)
 - 2) Delivery of database on dietary validation to P2 (D9)
- WP4:
 - 1) Dust samples delivered to P3 (part A) (D6)
 - 2) Delivery of dust samples to P3 for analysis of biological contaminants, Part B (D15)
- WP5:
 - 1) Delivery of fecal samples to P1 lab (D16)
- WP6:
 - 1) Database including data from questionnaires and blood samples in all centres (D20)
 - 2) Reports prepared on protective factors for allergy in children (D23)
 - 3) Overall evaluation report (D24)

Research activities during the fourth reporting period:

Analyses of the relation between farming characteristics and childhood allergy have been performed. A reporting of preliminary results was done at the project meeting in Basel.

Partner 5: Children's Hospital Salzburg; Austria

Principal investigator: Josef Riedler, MD, PhD
 Scientific staff: Ellen Üblagger, MD
 Waltraud Eder, MD

Objectives

P5, together with P4, is responsible for the design and implementation of WP2 as well as for managing the cross-sectional study in Austria and for participation in the overall analysis and reporting (WP 6).

Deliverables

- WP1:
 - 1) Protocol for selection of study subjects, local section (D1)
 - 2) Delivery of database including results from questionnaire study to P1 (D7)
- WP2:
 - 1) Questionnaire developed for assessment of atopy (D4)
 - 2) Protocol developed for bronchial challenge test (D5)
 - 3) Report prepared from bronchial challenge test (D11)
 - 4) Delivery of blood samples to P1 lab (D12)
- WP3
 - 1) Delivery of database on exposure to P1 (D8)
 - 2) Delivery of database on dietary validation to P2 (D9)
- WP4:
 - 1) Dust samples delivered to P3 (part A) (D6)
 - 2) Delivery of dust samples to P3 for analysis of biological contaminants, Part B (D15)
- WP5:
 - 1) Delivery of fecal samples to P1 lab (D16)
- WP6:
 - 1) Database including data from questionnaires and blood samples in all centres (D20)
 - 2) Reports prepared on protective factors for allergy in children (D23)
 - 3) Overall evaluation report (D24)

Research activities during the fourth reporting period:

Analyses were performed of the data from the bronchial challenge tests and a manuscript based on the results was prepared.

4: Technological Implementation Plan

Description of project

EC PROGRAMME:	LIFE QUALITY
PROJECT TITLE:	Prevention of allergy - risk factors for sensitisation in children related to farming and anthroposophic life style.
ACRONYM:	PARSIFAL
PROGRAMME TYPE:	5th FWP (Fifth Framework Programme)
CONTRACT NUMBER:	QLK4-CT-1999-01391
PROJECT WEB SITE (if any):	
START DATE:	01 Feb 2000
END DATE:	31 Jan 2004
COORDINATOR DETAILS:	Name: Göran Pershagen Organisation: Institute of Environmental Medicine, Karolinska Institute Address: Box 210, 171 77 Stockholm, Sweden Telephone: +46-8-524 874 60 E-mail: Goran.Pershagen@imm.ki.se

PARTNERS NAME:
Institute for Social and Preventive Medicine, University of Basel, Charlotte Braun-Fahrländer Institute for Risk Assessment Sciences, Utrecht University, Bert Brunekreef Dr von Haunersche Kinderklinik, Erika von Mutius Children's Hospital, Department of Paediatric Pulmonology & allergology, Josef Riedler

Commission Officer Name:	Ana Nieto
---------------------------------	-----------

Executive summary

Original research objectives

The ultimate aim of the project is to strengthen the basis for effective prevention of allergy in children, which has reached epidemic proportions in Europe and elsewhere in recent decades. It focuses on farming and anthroposophic children, two groups with a low prevalence of atopic diseases and sensitisation, but for which specific protective factors have not yet been identified. Specific objectives include assessment of the role of certain environmental and life style factors for the development of allergy in children, such as diet, vaccinations, infections and animal contact. In addition, the influence by indoor microbial contaminants and the intestinal microflora is studied. It should be emphasised that farming and anthroposophic children are focused for reasons of efficiency to obtain suitable distributions of exposure and that identification of important protective determinants of atopy has relevance for the general population. Scientific approach: Initially, this project will identify children in farming and anthroposophic families in five European countries: Austria, Germany, Netherlands, Sweden and Switzerland. Children are investigated according to a common protocol among farmers, families with an anthroposophic life style and controls. A questionnaire survey is carried out among the children to assess certain exposures, such as diet, animal contact, and history of infections and vaccinations, as well as atopic manifestations. The methodology will largely be based on internationally validated instruments and techniques. In addition, validations will be performed within the project of bronchial hyperreactivity and dietary history. Determinations are made in a central laboratory of biological contaminants in stables and other indoor environments, allergen specific IgE-levels in serum and intestinal microflora, respectively. The overall analyses and evaluation is aimed at identification of environmental and life style factors explaining the lower rate of allergy in children of farmers and in anthroposophic communities as well as to indicate effective strategies for prevention.

Expected deliverables

In order to test the hypothesis we included children, aged 5-13, from farm families, children with an anthroposophic lifestyle and their respective reference groups in Austria, Germany, Holland, Sweden and Switzerland. The final study population consisted of 14893 children, 19% farm children, 37% farm references, 31% Steiner school children and 14% Steiner references. A questionnaire was distributed to the parents to collect information on environmental exposures, life style, socioeconomic conditions, history of infections, diet, contact with animals, and on symptoms of bronchial asthma, allergic rhinoconjunctivitis, food allergy/urticaria and atopic eczema. One third of the children left blood samples, where allergen-specific IgE was measured against common inhalant and food allergens. Further dust samples were examined regarding certain microbial contaminants in the farm and anthroposophic environment in order to study the possible protective effect from this exposure. To assess the micro flora of the gut in relation to certain exposure characteristics, circa faecal samples from about 400 children were collected and analysed.

Project's actual outcome

There is a significantly lower prevalence of both subjective and objective markers of atopic disease when comparing the farm children and their reference group as well as when comparing the Steiner school group and their reference group. The protective effect of farming is found for all of the studied atopy outcomes, both self-reported, as for example current hay fever symptoms, wheezing, atopic eczema and asthma, and objective in the form of sensitisation (serum-IgE >0.35 kU/l) when studying all countries together. The risk of having current hay fever or being sensitised is only about 50% (OR 0.50 (95 CI 0.38-0.65) and OR 0.53 (95 CI 0.41-0.68), respectively for the farm children compared to their references. In the German subset the results look quite the same as for all the children, whereas the protective effect among farm children in the other countries appears consistent mostly for hay fever and sensitisation. The protective effect

of the anthroposophic life style is found for all of the studied outcomes except for current wheezing. For example the OR for having current hay fever or being sensitised were 0.71 (95 CI 0.57-0.88) and 0.75 (95 CI 0.59-0.95), respectively for the Steiner school children compared to their references. Also here there were some differences between the countries. Hardly any effect from the anthroposophic life style is found in the Austrian sub sample, and in Switzerland a clear lower risk in the Steiner group was seen only for sensitisation. Consistent protective effect of the anthroposophic life style is found primarily in Germany, the Netherlands and Sweden. A second aim of the study was to assess the possible role of certain environmental and lifestyle factors for the protective effect of the farming and Steiner life style. The analyses are still in progress and the results presented here should be considered preliminary. For the farm children, we found that children living on farms with pigs and farm children who have daily visits to the barn and those who have consumed farm milk during the first year or consume home made dairy products generally have lower risks of having the atopy outcomes. Few of the other studied farming characteristics seem to show consistent effects. Protective factors associated with an anthroposophic lifestyle might be the restricted use of antibiotics, antipyretics and the frequent consumption of organic/biodynamic food. There was an increased risk for asthma, atopic eczema and current wheezing associated with first use of antibiotics during 1st year of life. Atopic eczema was also related to the use of antibiotics after 12 months of age. Use of antipyretics in 1st year of life was also associated with a higher risk for asthma. Furthermore, consumption of organic/biodynamic food was related to a lower risk for atopic eczema and hay fever symptoms. Furthermore, the first results from the house dust analyses demonstrate that farm children and - to a lesser extent - Steiner children, are exposed to higher levels of endotoxin, EPS and glucans, than their respective references. This may contribute to the lower prevalence of atopy in the studied subgroups. Further analyses studying the relationship between components in the dust samples and atopy and asthma are in progress. One potentially weak point in several studies is that the estimated prevalence of asthma is based on questionnaire data. However, in the PARSIFAL study we found no substantial differences in the association between Bronchial hyperresponsiveness (BHR) test to hypertonic saline and questionnaire information on 'asthma' and 'wheeze' among the farm children, Steiner school children, farm reference children and Steiner reference children. This suggests that the comparisons of symptom rates between the different groups in our study were valid. Preliminary analyses of the faecal samples suggest differences in the intestinal bacterial flora between Steiner children and their references, as well as a presence of certain bacteria in children from pig farms. In view of the limited information available on the quality of dietary information for children obtained via questionnaire we investigated validity and reproducibility of the dietary data in PARSIFAL. Overall we found moderate to acceptable validity and moderate to substantial reproducibility in the parental reports of their children's diet. The quality was considered sufficiently accurate for diet-disease analyses of most foods.

Broad dissemination and use intentions for the expected outputs

In conclusion the PARSIFAL-study confirms that there is a protective effect from a farming and anthroposophic lifestyle for the development of allergy. Extensive ongoing analyses of the material will further clarify the role of specific environmental and life style factors for this effect. When the final results are ready they will be used for preventive measures, which are essential for coping with raising prevalence of atopic disease. Dissemination of the results: A brochure about the project has been produced. Three scientific articles have already been submitted and many more articles are planned or in preparation. The results from the study will also be presented at scientific meetings and spread to international and national organisations engaged in allergy prevention.

Overview of all your main project results

No.	Self-descriptive title of the result	Category A, B or C*	Partner(s) owning the result(s) (referring in particular to specific patents, copyrights, etc.) & involved in their further use
-----	--------------------------------------	---------------------	---

*A: results usable outside the consortium / B: results usable within the consortium / C: non usable results

Quantified Data on the dissemination and use of the project results

Items about the dissemination and use of the project results (consolidated numbers)	Currently achieved quantity	Estimated future* quantity
Product innovations	0	0
Process innovations	0	0
New services (commercial)	0	0
New services (public)	0	0
New methods	0	0
Scientific breakthrough	0	1
Technical standards to which this project has contributed	0	0
EU regulations/directives to which this project has contributed	0	1
International regulations to which this project has contributed	0	1
PhDs generated by the project	0	3
Grantees/trainees including transnational exchange of personnel	1	2

* "Future" means expectations within the next 3 years following the end of the project

Comment on European Interest

Community added value and contribution to EU policies

European dimension of the problem

European surveys indicate that manifestations of allergic diseases are observed in one third of the general population. The prevalence of allergic rhinitis, allergic asthma and atopic dermatitis has increased in recent decades, particularly in children. Preventive measures are essential for coping with the problem; however, additional evidence is needed to identify the most relevant risk factors. This is particularly important in children since the atopic manifestations develop at an early age. In conclusion the PARSIFAL-study confirms that there is a protective effect from a farming and anthroposophic lifestyle for the development of allergy. Extensive ongoing analyses of the material will further clarify the role of specific environmental and life style factors for this effect. When the final results become available they will be used for preventive measures.

Contribution to developing S&T co-operation at international level. European added value

A brochure about the project has been produced. Three scientific articles have already been submitted and many more are planned. The results from the study will also be presented at scientific meetings and spread to organisations international and national organisations engaged in allergy prevention. The project has also strengthened the co-operation in the field of asthma-allergy research in Europe and the co-operation will continue also after the completion of the EU-funded study.

Contribution to policy design or implementation

The PARSIFAL-study confirms that there is a protective effect from a farming and anthroposophic lifestyle for the development of allergy. Extensive ongoing analyses of the material will further clarify the role of specific environmental and life style factors for this effect. When the final results become available they will be used for preventive measures, which are essential for coping with raising prevalence of atopic disease. The results from the study will also be presented at scientific meetings and spread to organisations international and national organisations engaged in allergy prevention.

Contribution to Community social objectives

Improving the quality of life in the Community:

See contribution to policy design and implementation (above).

Provision of appropriate incentives for monitoring and creating jobs in the Community (including use and development of skills):

-

Supporting sustainable development, preserving and/or enhancing the environment (including use/conservation of resources):

The role of the environment is of importance for many of the diseases in the modern society. The prevalence of allergic rhinitis, allergic asthma and atopic dermatitis has increased sharply in recent decades, particularly in children. However, the reasons behind this increase are still not elucidated. Preventive measures are essential for coping with the problem; however, additional evidence is needed to identify the most relevant risk factors. This is particularly important in children since the atopic manifestations develop at an early age.

Expected project impact (to be filled in by the project coordinator)

EU Policy Goals	I SCALE OF EXPECTED IMPACT OVER THE NEXT 10 YEARS -1 0 1 2 3	II other Not applicable to project Project Impact too difficult to estimate
1. Improved sustainable economic development and growth, competitiveness	1	
2. Improved employment	0	√
3. Improved quality of life and health and safety	2	
4. Improved education	0	√
5. Improved preservation and enhancement of the environment	0	√
6. Improved scientific and technological quality	2	√
7. Regulatory and legislative environment	1	
8. Other	1	

1. Economic development and growth, competitiveness	Scale of Expected Impacts over the next 10 years (2)	
	By Project End	After Project End
	-1 0 1 2 3	-1 0 1 2 3
a) Increased Turnover for project participants - national markets	0	0
b) Increased Turnover for project participants - international markets	0	0
c) Increased Productivity for project participants	0	0
d) Reduced costs for project participants	0	0
e) Improved output quality/high technology content	0	0

2. Employment	Scale of Expected Impacts over the next 10 years (2)	
	By Project End	After Project End
	-1 0 1 2 3	-1 0 1 2 3
a) Safeguarding of jobs	0	0
b) Net employment growth in projects participants staff	0	0
c) Net employment growth in customer and supply chains	0	0
d) Net employment growth in the European economy at large	0	0

3. Quality of Life and health and safety	Scale of Expected Impacts over the next 10 years (2)	
	By Project End	After Project End
	-1 0 1 2 3	-1 0 1 2 3
a) Improved health care	0	2
b) Improved food, nutrition	0	1
c) Improved safety (incl. consumers and workers safety)	0	1
d) Improved quality of life for the elderly and disabled	0	0
e) Improved life expectancy	0	0
f) Improved working conditions	0	0
g) Improved child care	0	2

h) Improved mobility of persons	0	0

4. Improved education	Scale of Expected Impacts over the next 10 years (2)	
	By Project End	After Project End
	-1 0 1 2 3	-1 0 1 2 3
a) Improved learning processes including lifelong learning	0	0
b) Development of new university curricula	0	0

5. Preservation and enhancement of the environment	Scale of Expected Impacts over the next 10 years (2)	
	By Project End	After Project End
	-1 0 1 2 3	-1 0 1 2 3
a) Improved prevention of emissions	0	0
b) Improved treatment of emissions	0	0
c) Improved preservation of natural resources and cultural heritage	0	0
d) Reduced energy consumption	0	0

6. S&T quality	Scale of Expected Impacts over the next 10 years (2)	
	By Project End	After Project End
	-1 0 1 2 3	-1 0 1 2 3
a) Production of new knowledge	1	2
b) Safeguarding or development of expertise in a research area	1	2
c) Acceleration of RTD, transfer or uptake	1	1
d) Enhance skills of RTD staff	1	1
e) Transfer expertise/know-how/technology	2	2
f) Improved access to knowledge-based networks	2	2
g) Identifying appropriate partners and expertise	2	2
h) Develop international S&T co-operation	2	2
i) Increased gender equality	0	0

7. Regulatory and legislative environment	Scale of Expected Impacts over the next 10 years (2) By Project End -1 0 1 2 3 After Project End -1 0 1 2 3	
a) Contribution to EU policy formulation	0	2
Contribution to EU policy implementation	0	1

8. Other (please specify)	Scale of Expected Impacts over the next 10 years (2) By Project End -1 0 1 2 3 After Project End -1 0 1 2 3	
	0	1
Description of Results		

STLL NO RESULT(S) FOR THIS PROJECT.

Exploitation plans

CONFIDENTIAL

I am the Co-ordinator of the above project, and confirm on behalf of the contracted Partners the information contained in this Technological Implementation Plan, and I authorise its public dissemination.

Signature:

Name:

Date:

Organisation:

5: Executive summary of the whole project

Contract no QLK4-CT-1999-01391

Reporting period: 1 Feb 2000 – 31 Jan 2004

Title: PARSIFAL

Background & Objectives: European surveys indicate that manifestations of allergic diseases are observed in one third of the general population. The prevalence of allergic rhinitis, allergic asthma and atopic eczema has increased in recent decades, particularly in children. Preventive measures are essential for coping with the problem; however, additional evidence is needed to identify the most relevant risk or protective factors. This is particularly important in children since the atopic manifestations develop at an early age.

The PARSIFAL project focuses on farm children and children with an anthroposophic lifestyle, because some earlier studies have shown a lower prevalence of atopic diseases and sensitisation in these groups. Specific protective factors had, however, not been identified, but proposed protective factors included animal contact and certain dietary factors. In the earlier studies it had been difficult to assess the role of specific exposure factors because of strong correlations between the factors. The aim of PARSIFAL-study has been to examine if the results from these earlier studies could be confirmed and if there are any differences among the possible protecting effects from these lifestyles in five different countries in Europe (Austria, Germany, Holland, Sweden and Switzerland). A further aim has been to assess the role of certain environmental and lifestyle factors for development of allergy in children.

Scientific achievements: We included children, aged 5-13, from farm families, children with an anthroposophic lifestyle and corresponding reference groups in Austria, Germany, Holland, Sweden and Switzerland. The final study population consisted of 14893 children, 19% farm children, 37% farm references, 31% Steiner school children and 14% Steiner references. A questionnaire was distributed to the parents to collect information on environmental exposures, life style, socioeconomic conditions, history of infections, diet, contact with animals, and on symptoms of bronchial asthma, allergic rhinoconjunctivitis, food allergy/urticaria and atopic eczema. One third of the children left blood samples for measurement of allergen-specific IgE against common inhalant and food allergens. Further dust samples were examined regarding certain microbial contaminants and allergens in the farm and anthroposophic environment. To assess the microflora of the gut in relation to certain exposure characteristics, faecal samples from about 400 children were collected.

Main deliverables: There is a significantly lower prevalence of both subjective and objective markers of atopic disease when comparing the farm children and their reference group as well as when comparing the Steiner school group and their reference group.

The protective effect of farming is found for all of the studied atopy outcomes, both self-reported, as for example current hay fever symptoms, wheezing, atopic eczema and asthma, and objective in the form of sensitisation (serum-IgE ≥ 0.35 kU/l) when studying all countries together. The risk of having current hay fever or being sensitised is only about 50% (OR 0.50 (95 CI 0.38-0.65) and OR 0.53 (95 CI 0.41-0.68), respectively, for the farm children compared to their references. In the German subset the results look quite the same as for all the children, whereas the protective effect among farm children in the other countries appears consistent mostly for hay fever and sensitisation.

The protective effect of the anthroposophic life style is found for all of the studied outcomes except for current wheezing. For example the OR for having current hay fever or being sensitised were 0.71 (95 CI 0.57-0.88) and 0.75 (95 CI 0.59-0.95), respectively, for the Steiner school children compared to their references. Also here there were some differences between the countries. Hardly any effect from the anthroposophic life style is found in the Austrian sub

sample, and in Switzerland a clear lower risk in the Steiner group was seen only for sensitisation. Consistent protective effect of the anthroposophic life style is found primarily in Germany, the Netherlands and Sweden.

A second aim of the study was to assess the possible role of certain environmental and lifestyle factors for the protective effect of the farming and Steiner life style. The analyses are still in progress and the results presented here should be considered preliminary. For the farm children, we found that children living on farms with pigs and farm children who have daily visits to the barn and those who consumed farm milk during the first year or home made dairy products generally have lower risks of the atopy outcomes. Few of the other studied farming characteristics seem to show consistent effects. Protective factors associated with an anthroposophic lifestyle might be the restricted use of antibiotics, antipyretics and the frequent consumption of organic/biodynamic food. There was an increased risk for asthma, atopic eczema and current wheezing associated with first use of antibiotics during 1st year of life. Atopic eczema was also related to the use of antibiotics after 12 months of age. Use of antipyretics in 1st year of life was also associated with a higher risk for asthma. Furthermore, consumption of organic/biodynamic food was related to a lower risk for atopic eczema and hay fever symptoms.

The first results from the house dust analyses demonstrate that farm children and - to a lesser extent - Steiner children, are exposed to higher levels of endotoxin, EPS and glucans, than their respective references. This may contribute to the lower prevalence of atopy. Further analyses of the relationship between components in the dust samples and atopy and asthma are in progress.

One potentially weak point in several studies is that the estimated prevalence of asthma is based on questionnaire data. However, in the PARSIFAL study we found no substantial differences in the association between Bronchial hyperresponsiveness (BHR) test to hypertonic saline and questionnaire information on 'asthma' and 'wheeze' among the farm children, Steiner school children, farm reference children and Steiner reference children. This suggests that the comparisons of symptom rates between the different groups were valid.

Preliminary analyses of the faecal samples suggest differences in the intestinal bacterial flora between Steiner children and their references, as well as a presence of certain bacteria in children from pig farms.

In view of the limited information available on the quality of dietary information for children obtained via questionnaire we investigated the validity and reproducibility of the dietary data in PARSIFAL. Overall we found moderate to acceptable validity and moderate to substantial reproducibility in the parental reports of their children's diet. The quality was considered sufficiently accurate for diet-disease analyses of most foods.

Socio-economic relevance and conclusions: In conclusion the PARSIFAL-study confirms that there is a protective effect from a farming and anthroposophic lifestyle for the development of allergy in children. Extensive ongoing analyses will further clarify the role of specific environmental and life style factors for this effect. When results become available they will be used as a basis for preventive measures.

Dissemination of the results: A brochure about the project has been produced. Three scientific articles have already been submitted for publication and many more are in preparation. The results from the study will also be presented at scientific meetings and spread to international and national organisations engaged in allergy prevention. Further there will be several PhD and master theses based on the findings from the project.

Keywords: Allergy, anthroposophic lifestyle, asthma, children, diet, environmental factors, farming lifestyle, prevention, sensitisation.

6: Detailed report related to overall project duration

6.1 Background

European surveys indicate that manifestations of allergic diseases are observed in one third of the general population. The prevalence of allergic rhinitis, allergic asthma and atopic dermatitis has increased in recent decades, particularly in children (Burney et al. 1990; Braback et al. 1994; 1998). The total costs for the major allergic diseases in Europe are estimated to 10 billion Euro for direct costs and 19 billion Euro for indirect costs (European Allergy White Paper (1997). Preventive measures are essential for coping with the problem; however, additional evidence is needed to identify the most relevant risk factors. This is particularly important in children since the atopic manifestations develop at an early age.

The PARSIFAL project focuses on two groups of children with a relatively low prevalence of atopic diseases and sensitisation. When the study was designed it had been documented that children of farmers had a lower prevalence of atopic diseases, primarily with regard to asthma and/or rhinoconjunctivitis (Braun-Fahrlander et al. 1999; Riedler et al. 2000; Von Ehrenstein et al. 2000; Klintberg et al. 2001). Furthermore, a previous study showed that children in an anthroposophic community had a markedly reduced risk of atopic diseases and IgE-sensitisation (Alm et al. 1999). Specific protective factors had, however, not been identified, but proposed protective factors included animal contact, certain dietary factors and infections. In the earlier studies it had been difficult to assess the role of specific exposure factors because of strong correlations between the factors. In PARSIFAL farming and anthroposophic children are targeted for reasons of efficiency to obtain suitable distributions of exposure but identification of important determinants of atopy has clear relevance also for the general population.

6.2 Objectives

The overall aim of the project was to assess the role of certain environmental and lifestyle factors for development of allergy in children. A novelty in the approach is to focus primarily on factors offering protection in relation to allergy. Reduced exposure to such factors may contribute to explaining the rise in allergy in many western countries in recent decades. Furthermore, identification of protective factors is probably crucial for successful prevention.

The specific hypotheses to be tested include whether the risk of allergic disease or atopy is related to certain dietary components, such as fermented vegetables containing live lactobacilli, antioxidants or lipids. Furthermore, the role of specific infections and vaccinations is assessed. Protective effects of animal contact and certain microbial contaminants are also evaluated. The intestinal microflora is monitored in a subgroup to determine its relation to certain exposures.

The specific research questions were:

- 1) Is the prevalence of atopic diseases lower in farm children than in non-farm children?
- 2) If there are any differences, are they consistent across different countries in Central and Northern Europe?
- 3) Is the prevalence of atopic diseases lower in Steiner school children than in non-Steiner school children?

- 4) If there are any differences, are they consistent across different countries in Central and Northern Europe?
- 5) Which specific factors protect farm children from developing atopic diseases, e.g. farm animal contacts, certain dietary components?
- 6) Which specific factors protect Steiner school children from developing atopic diseases, e.g. certain dietary components, such as fermented vegetables, specific infections and vaccinations?
- 7) Which is the possible protective effect of certain microbial contaminants in the farm and anthroposophic environment? Does the exposure of microbial contaminants differ between different groups and countries in the study?
- 8) Which is relationship between certain exposures and the intestinal microflora?
- 9) Is the questionnaire information on asthma and wheeze valid when the linguistically and culturally heterogeneous populations are compared?
- 10) What is the validity of the dietary information provided by the parents as proxies for their child?

6.3 Subjects and Methods

Subjects

The aim of the PARSIFAL project was to include children of farmers and in anthroposophic communities as well as references to the respective groups in five European countries: Austria, Germany, the Netherlands, Sweden and Switzerland. A total of around 6000 children aged 6 to 12 years were planned to be invited to answer a questionnaire and to leave blood samples. A non-response to the questionnaire of about 20% was assumed and the final study size for combined analysis was estimated at approximately 1 600 children each among children of farmers, in anthroposophic communities and among references.

The selection of the children and the fieldwork are described below. For a more thorough description of the selection and the fieldwork in the respective countries, see appendix 1.

Children with an anthroposophic life style

Children were selected from classes in Steiner schools with children of appropriate age. In Germany and Switzerland an over-sampling was done, because it was thought to be quite common that “non-anthroposophic” parents also send their children to Steiner schools and the aim was to get enough children with a “genuine” anthroposophic lifestyle in the study. After a first sampling seven schools with a low participation rate, considered to be due to school disinterest rather than refusal by individuals, were excluded in Germany. After this first exclusion a total of 6978 children in the five participating countries were invited to participate, and a total of 4678 filled in and returned the questionnaire (participation rate 67%).

Steiner:

Austria: Steiner schools

Germany: Steiner schools. It was estimated that approx. 15 percent of children going to Steiner schools in Germany would have a “genuine” anthroposophic lifestyle, so the aim was to over-sample, and recruit 4 200 children. Seven schools with a low participation rate were then excluded.

The NL: Steiner schools located in the vicinity of the Dutch study centre and in areas with large Steiner populations.

Sweden: Steiner schools in areas with many Steiner people. All pupils of the right age were selected.

Switzerland: Steiner schools. Some oversampling was performed, because it is quite common that “non-anthroposophic” parents also send their children to Steiner schools

References to the children with an anthroposophic life style

Children were selected from schools in the vicinity of the Steiner schools and in the same type of surroundings, e.g. if the Steiner school was in a suburb the reference school was also in a suburb, if the Steiner school was in a rural area, the reference school was also in a rural area. After a first sampling one school with a low participation rate was excluded in Germany. After this first exclusion a total of 2958 children in the five participating countries were invited to participate, and a total of 2055 filled in and returned the questionnaire (participation rate 69%).

Steiner References:

Austria: Schools in the vicinity of the Steiner schools

Germany: Schools in the vicinity of the Steiner schools. One school with a low participation rate was then excluded.

The NL: Schools in the vicinity of the Steiner schools

Sweden: Schools in the vicinity of the Steiner schools.

Switzerland: Schools in the vicinity of the Steiner schools

Farm children

The selection process to recruit farm children differed between the countries. In Austria children of farmers had been selected by teachers with a good knowledge of the pupils and their parents

in an earlier study. The same method was used again; so all pupils to farmers in some classes were selected by their teachers. In Germany, the Netherlands and Switzerland schools in rural areas known to have a rather high percentage of farmers were selected and the questionnaires were distributed to all the children in some classes without knowing if they were children to farmers or not. In Sweden farmers with children in the appropriate age were selected from the Farming registry held by the National Bureau of Statistics. In view of the methodologies for selection of the study subjects in some countries it was not possible to calculate a response rate for the farm children and their reference group separately. However, a combined response rate for the farmer and farmer reference group was 70% (8402/11969).

Farm children:

Austria: Selected by teachers in schools in rural areas.

Germany: Schools in rural areas. When selected status as farm or not farm child was not known.

The NL: Schools with a percentage of farmers >10%. When selected status as farm or not farm child was not known.

Sweden: Farmers with children in the appropriate age selected from the Farming registry held by the National Bureau of Statistics.

Switzerland: Schools in a rural area with a percentage of farmers of 15-20%. When selected status as farm or not farm child was not known from some schools only children to farmer through a farming organisation. See table 1 for the exact numbers.

Farm reference children

In Germany, the Netherlands, and Switzerland the farm reference children and the farm children were selected at the same time, without knowing if the family was a farm family or not, as described above. In Austria the farm reference children were randomly selected from the same schools as the farm children, among children not designated as farm children by their teachers, matched for age and gender.

In Sweden families with children in the appropriate age in the same area as the farm children were randomly selected from the population registries held by the National Bureau of Statistics. Due to the methods of recruitment in some countries it is not possible to calculate response rate for the farm reference group separately (see above).

Farm reference children:

Austria: The reference children were chosen from the same schools as the farm children, randomly selected from the same schools as the farm children, from the children not designated as farm children by their teachers, matched for age and gender.

Germany: Chosen from the same schools as farmers. When selected status as farm or not farm child was not known.

The NL: Chosen from the same schools as farmers. When selected status as farm or not farm child was not known.

Sweden: Randomly selected families with children in the appropriate age in the same area as the farmers, from the population registries held by the National Bureau of Statistics.

Switzerland: Schools in a rural area with a percentage of farmers of 15-20%. When selected status as farm child or not was not known.

In total 21 905 children were invited to take part in the study and 15137 children answered the questionnaire (participation rate 69%). An overview of the participation rates in the different exposure groups and in the different countries is given in table 1. The participation rate for the questionnaire differed between the countries, from about 80% in Austria and Switzerland, 75% in Germany 71% in Sweden and 50% in the Netherlands. However, the participation rates did not differ much between the different groups in each country, decreasing the risk of selection bias.

Table 1. Number of selected and included children and participation rate for the questionnaire and blood samples.

Country	No of selected children		No of children in database, with a questionnaire	Participation rate (%)	Selected for blood-sampling	No of blood-samples in database	Part. Rate(%) Blood samples
Austria							
Unknown group			2			2	
Farm children	400		342	85.5	226	214	94.7
Farm References	241		209	86.7	138	135	97.8
Steiner school children	629		461	73.3	247	233	94.3
Steiner references	319		268	84.0	195	175	89.7
Total	1589		1282	80.7	806	759	94.2
Germany							
Unknown group						5	
Farm children			985		968 ³	623	64.4
Farm References		5773 ¹	3365	75.4	150 ³	147	98.0
Steiner school children	2471 ²		1814	73.4	280 ³	245	87.5
Steiner references	996 ²		799	80.2	150 ³	139	92.7
Total	9240		6963	75.4	1548	1159	74.9
The Netherlands							
Unknown group							
Farm children	300		527		124	102	82.3
Farm References		3034 ¹	1242	53.1	221	181	81.9
Steiner school children	2157		1038	48.1	222	162	73.0
Steiner references	912		423	46.4	124	107	86.3
Total	6403		3230	50.4	691	552	79.9

Country	No of selected children		No of children in database, with a questionnaire	Part.rate(%)	Selected for blood-sampling	No of blood-samples in database	Part.rate(%) Blood samples
Sweden							
Unknown group						3	
Farm children	500		330	66.0	293	253	86.3
Farm References	250		172	68.8	116	105	90.5
Steiner school children	550		442	80.4	397	353	88.9
Steiner references	250		165	66.0	138	122	88.4
Total	1550		1109	71.5	944	836	88.6
Switzerland							
Unknown group							
Farm children	410 + 48	1013 ¹	692	83.6	254 ³	222	87.4
Farm References			538		163 ³	150	
Steiner school children	1171		923	78.8	304	252	82.9
Steiner references	481		400	83.2	144 ³	119	82.6
Total	3123		2553	81.7	865	743	85.9
Total (all centres)							
Unknown group			2			10	
Farm children	1658	9820	2876	70.2	1865	1414	75.8
Farm References	491		5526		788	718	91.1
Steiner school children	6978		4678	67.0	1450	1245	85.9
Steiner references	2958		2055	69.5	751	662	88.1
Total (all centres)	21 905		15137	69.1	4854	4049	83.4

1. Farm children and farm references selected from the same schools and then divided into farmers and references.

2. An exclusion of seven schools for the Steiner school children and one school for the Steiner references due to a very low overall participation rates (see above).

3. Randomly selected and 28 extra wheezers from Germany and 38 extra wheezers (see chapter about BHR for definition) from Switzerland included.

A total of 15137 children answered the questionnaire. Table 2 shows the number of children who participated in the different examinations. As can be seen 46% of the children with a valid questionnaire are German. However, there is a more even distribution of the children between the countries for the different clinical examinations.

Table 2. Children with a completed questionnaire and the various clinical examinations, divided by centre and group.

		No. of included children	No. of blood	No. of sock	No. of ALK	No. of feaces	No. of BHR
Austria		2	2				
	Farm	342	214	163	50	46	35
	Farm ref	209	135	107	13	12	32
	Steiner	461	233	256	25	24	52
	Steiner ref	268	175	132	12	13	41
	Total	1282	759	658	100	95	160
Germany			5				2
	Farm	985	623	352	53	48	51
	Farm ref	3365	147	130	12	10	52
	Steiner	1814	245	211	27	20	54
	Steiner ref	799	139	118	18	10	52
	Total	6963	1159	811	110	88	211
Holland	Farm	527	102	87	68	50	36
	Farm ref	1242	181	138	13	6	38
	Steiner	1038	162	255	25	19	48
	Steiner ref	423	107	99	13	10	44
	Total	3230	552	579	119	85	166
Sweden			3				
	Farm	330	253	232	50	38	44
	Farm ref	172	105	85	13	11	22
	Steiner	442	353	309	25	21	61
	Steiner ref	165	122	109	12	8	30
	Total	1109	836	735	100	78	157
Switzerland	Farm	692	222	221	50	34	45
	Farm ref	538	150	159	13	9	46
	Steiner	923	252	348	26	21	63
	Steiner ref	400	119	114	12	8	45
	Total	2553	743	842	101	72	199
All centres		2	10				2
	Farm	2876	1414	1055	271	216	211
	Farm ref	5526	718	619	64	48	190
	Steiner	4678	1245	1379	128	105	278
	Steiner ref	2055	662	572	67	49	212
	Total	15137	4049	3625	530	418	893

Table 3 includes only the 14 893 children with a completed questionnaire and within the right age range (5-13 years) (236 children excluded) and not missing on sex (seven children excluded) or missing on group (one child excluded). Some of the children who originally were classified as farmer references (12 children) and Steiner references (22) had answered that they actually live on a farm and that their family runs the farm. Hence these children have been recoded as farmers. Of the children originally classified as farm children twenty-three had answered that they do not live on a farm and that the families do not run a farm. These children have been recoded as farm references. After this recoding, 14893 children were left and used in all following tables and analyses in this report. Of the children 19% were farm children, 37% farm references, 31% Steiner school children and 14% Steiner references. Almost half of the children are German (46%), 21% Dutch, 17% Swiss and around 8% from Austria and Sweden respectively.

Table 3. Number of children who have a questionnaire, within the right age range (5-13 years), not missing on sex or missing on group, subdivided by centre and group.

	Farmer		Farmer ref		Steiner		Steiner ref		All groups	
	n	%	n	%	n	%	n	%	n	%
Austria	328	11.6	202	3.7	447	9.7	255	12.6	1232	8.3
Germany	964	34.1	3305	60.8	1785	38.8	789	39.0	6843	45.9
Holland	517	18.3	1225	22.5	1019	22.1	420	20.8	3181	21.4
Sweden	330	11.7	172	3.2	442	9.6	165	8.2	1109	7.4
Switzerland	684	24.2	536	9.9	913	19.8	395	19.5	2528	17.0
All centres (total)	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

Questionnaire

A questionnaire was distributed to collect information on environmental exposures, life style, socioeconomic conditions, history of infections, diet, contact with animals, as well as on symptoms of bronchial asthma, allergic rhinoconjunctivitis, food allergy/urticaria and atopic dermatitis.

Most of the questions on exposures associated with the home environment (bedroom sharing, pets, smoking, cooking and heating fuel, air conditioning, damp spots and visible moulds, floor covering, insulation), the family environment (number of younger and older siblings, family history of diseases, child's personal history of infections and immunisation, birth weight, breastfeeding) and socio-economic conditions were based on the internationally validated and translated ISAAC phase II questions (ISAAC (1998)) and questions from the BAMSE (Wickman et al. 2002) studies. Questions on exposures and lifestyle factors related to living on a farm (contact to farm animals, time spent in barns and with agricultural activities) were largely based on questionnaires developed for recent studies in Switzerland, Germany and Austria (Riedler et al. 2001). Information on factors associated with anthroposophic lifestyle was based on questions developed for a recent Swedish study (Alm et al. 1999).

Long-term diet was assessed using semi quantitative food frequency questions. The questions were designed to specifically include food items that discriminate between our study populations (children of farming families and anthroposophic families) and the general population. The mother of the child included in the study was asked to provide the relevant dietary information on the child. The food questions were then validated in a subsample of representative families with a 24-h dietary recall and some similar food frequency questionnaire administered by telephone (see 24-h-diet recall).

To assess prevalence of atopic diseases, the questionnaire distributed to the parents also included questions on symptoms and diagnosis of asthma, hay fever and atopic dermatitis according to the internationally standardised ISAAC protocol. The ISAAC questions on asthma have been validated with respect to bronchial hyper responsiveness (see BHR below).

The questions were originally drafted in German (except for the questions regarding anthroposophic life style which were originally written in Swedish and drafted in English) and then translated and back translated into the other languages. The questionnaires were printed in October 2000.

In the Netherlands the questionnaire was sent to print first, before some final modifications, and therefore differed slightly from the other countries (see the Netherlands fieldwork documentation in appendix 1 for full information).

The questionnaires were distributed and collected from October 2000 to November 2001 during different periods in the different countries. In Austria and Sweden the questionnaires were entered twice manually and in the Netherlands and in Switzerland professional data entry institutes were used.

Austria: Distributed in schools in the beginning of 2001 and collected two weeks later. Data entered manually from June until September 2001.

Germany: Time for the collection ? Entered in which way?

The Netherlands: Distributed in schools and collected in the schools. Time for collection between October 2000 and May 2002 Data entered by a professional data entry institute.

Sweden: Distributed in the end of October 2000, collected Nov 2000- Nov-2001. Entered twice manually.

Switzerland: Distribution and collection in January – April 2001. Professional data entry institute.

The data entry error was calculated in some of the countries and was in the Netherlands 0.4%, in Sweden 0.1% and in Switzerland 0.17%. For full information see the respective country fieldwork documentations (appendix 1).

Clinical examination and blood sampling

The clinical examination included blood samples, measurement of weight and height, and collection of information about the child's vaccinations. The selection criteria differed between the different countries, as explained below, but altogether there were 4049 blood samples were analysed for allergen-specific IgE (see table 2).

The blood samples were taken according to a common study protocol. The children were offered the use of an anaesthetic lotion (EMLA®). Approximately, 10 ml (Serum tubes) was drawn for measurement of allergen-specific IgE.

All samples were screened with a mixture of common inhalant (Phadiatop) and food allergens (fx5) (Pharmacia CAP System, Pharmacia Diagnostics AB, Uppsala, Sweden). Sera, which were scored positive in Phadiatop, were further analysed separately against the dust mites *Dermatophagoides pteronyssinus* and *Lepidoglyphus destructor*, grass pollen, tree pollen, cat and horse epithelium. All CAP-analyses were performed centrally at the Department of Clinical Immunology at the Karolinska University Hospital, Stockholm, Sweden.

As shown in table 1 not all children who answered the questionnaire were invited to the blood sampling, due to the fact that some of them had not given informed consent. In Germany and Switzerland it was not necessary to include all children who had given informed consent to fulfil the planned number of blood samples. Not all of the invited children left a blood sample and

reasons for not obtaining a blood sample during the clinical examination was: the nurses could not draw a blood sample, the child was not available at the day of blood sampling or the child refused consent.

Selection of children for clinical examination and blood samples:

Austria: All children who had consented to blood sampling, 759 blood samples are available in the database

Germany: *Farm children:* All children who consented to blood sampling, (635/968 or 65.6%). *Farm references:* 150 children randomly selected of these who had consented to blood sampling. Eventually 139/150 (92.7 %) blood samples were obtained. *Steiner school children:* Children with a supposedly “genuine” anthroposophic life style were chosen. They were selected using a “Steiner criterium” based on questions 10-13, a-c, in the questionnaire, which was fulfilled if:

- o the parents decided before the birth of the child that the child will visit a Steiner school *and*
- o the child was sent to a Steiner school because of the anthroposophic education *and*
- o at least one of the parents had an anthroposophic view of life *and*
- o the parents got interested in anthroposophy before the birth of the child *and at least one of the following points:*
- o there was some joint evening ritual in the family with singing and praying
- o the families paid attention to the colouring of the rooms (e.g. with special colours)
- o the family had a “seasonal table” within the flat

694 children fulfilled these criteria and 368 (53%) consented in all measurements. Of these children all wheezers (n=29) and a random sub sample of non-wheezers (n=251) were selected for blood sampling, eventually blood samples were collected from 243/280 (86.8 %) of selected children. *Steiner references:* All wheezers (n=21) and a random sub sample of non-wheezers (n=129) were selected; eventually blood samples were collected from 144/150 (96.0 %) selected children.

Netherlands: Almost all children whose parents consented to blood sampling were selected for this part. Only a few too young (<6) or too old (>11) children were excluded. For IgE, 552 samples were obtained.

Sweden: All subjects, who had answered the questionnaire and not already declined add-on investigations when they returned the questionnaire, were invited to the clinical examination and blood sampling. 155 of the children (41 Steiners, 33 farmers, 27 Steiner references and 54 farmer references) had declined all participation in clinical examinations and 10 (4 farmers, 4 Steiners, 2 farmer references) only blood sampling. Of the remaining 944 invited children, 852 came to the clinical examination and 836 provided a blood sample.

Switzerland: A random selection of all children who accepted blood sampling and dust sampling, and 38 extra wheezers. From the totally 865 selected children 743 (86%) serum samples could be collected and frozen for further analysis.

The sampling procedure used in Germany causes potential problems with respect to the representativeness of the Steiner and Steiner reference children. For both groups blood sampling was performed for all children with wheeze but only in a random subsample of non-wheezers. Thus, both of these subgroups are - to a different extent – slightly enriched with wheezers with respect to blood samples. In the Steiner group the prevalence of wheeze is 7.9 % among the Steiner school children who were invited to the blood sampling and who had consented to all measurements, and 10.4 % among the Steiner school children with obtained blood samples. In the Steiner reference group the prevalence of wheeze is 6.6 % among those consenting to all measurements, and 14 % among those with obtained blood samples. However, by randomly excluding 7 wheezers in the Steiner school children group and 21 wheezers in the Steiner reference group, the random proportion of sampling has been regained.

In Switzerland too, blood samples were not only taken from randomly selected children. As in Germany some extra wheezers (32) were also included. The “extra wheezers” from Germany and Switzerland are not included in the following tables.

In table 4 the final numbers of IgE-analyses included in the further analyses are shown. Here only the children of the right age (5- 13 years old) and the children randomly selected to be in the blood analyses group in the German and Swiss groups as explained above are included.

Table 4. Final number of children used in analyses who have IgE blood samples, distributed by centre and group.

	Farmer		Farmer ref		Steiner		Steiner ref		All groups	
	n	%	n	%	n	%	n	%	n	%
Austria	208	15.0	132	18.8	228	19.0	172	27.1	740	18.9
Germany	614	44.3	144	20.5	236	19.7	125	19.7	1119	28.5
Holland	102	7.4	179	25.5	162	13.5	107	16.9	550	14.0
Sweden	250	18.0	104	14.8	352	29.3	122	19.2	828	21.1
Switzerland	212	15.3	142	20.3	223	18.6	108	17.0	685	17.5
All centres (total)	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0

As explained above, it should also be noted that blood sampling in Germany among the Steiner children was only performed in the Steiner school children with a “genuine” anthroposophic lifestyle. This must be taken into account when comparing the German blood data to the other centres.

The calculations of the participation rate for the blood sampling are shown in table 5. The response rate differed between the countries, from 54% in Sweden to 9% in the Netherlands. Reasons for the low participation rate in the Netherlands could be the outbreaks of animal diseases, and the outbreak of Foot and Mouth Disease during the PARSIFAL recruitment phase in particular, might have negatively influenced the response rates among Dutch farmers. The new regulations of the Dutch Medical Ethical Committees have also become more stringent. Detailed information on medical examinations, including risk evaluations, and consent conditions are required, which might have scared people off.

Table 5. Participation rate for the blood sampling, divided by country.

Country	No. of selected children	Consent for blood sample (IgE)	Selected for blood sample	Blood samples	Part.rate (%)
Austria	1589	806	806	759	47,8
Germany	9240	5045	1548	1159	40,9
Holland	6403	691	691	552	8,6*
Sweden	1550	944	944	836	53,9
Switzerland	3123	1302	865	743	35,8
Sum	21905	8788	4854	4049	33,5%

*Holland: Not all Steiner school children were asked for consent to the medical/dust parts of the project, because the questionnaires were distributed first, and consent forms after that in Steiner children (see page 2 of Dutch fieldwork documentation for further information). Of the 1038 returned questionnaires from Steiner school children, only 888 were asked for consent, because the other 150 children were either too young/old, or from one school which did not take part in the other investigations, or parents indicated that they did not want to receive further information. If this is taken into account, the response rate for blood sampling would not be 552/6403=8.6%, but 552/6253=8.8%.

In the Netherlands comparisons of atopic disease in the PARSIFAL study have also been done with the ISAAC-II-study (table 6), which indicate that no obvious selection bias has occurred regarding atopic disease status.

Table 6. Comparison of prevalence of atopic disease in the ISAAC-II-study and PARSIFAL

	ISAAC (n=2509)	PARSIFAL (N=3184)
Current atopic dermatitis	14.8%	12.7%
Hay fever symptoms, 12 mo	5.3%*	5.2%
IgE (Phadiatop)	27.9%	28.0%
Wheeze, 12 mo**	9.4%	9.1%

* not in paper of Janssen, but in paper of Aarts et al (not published)

**Prevalence of current wheeze (12 months) in study in 1990 (N=6109) 10.2%.

In most of the following analyses the cut-off point for atopic sensitisation is >0.35 kU/litre (for either Phadiatop or Fx5).

Dust samples

Dust sample socks

It was planned to collect dust from approximately 1200 children from each country and the selection criteria differed slightly between the different countries. Dust sample socks, instructions for the dust sampling and a short questionnaire with questions about type of carpets and mattresses were distributed via mail to the families, according to a common protocol. The parents collected the dust samples from their child's mattress and the living room floor. The dust samples were then returned via mail or at the time for the clinical examination. The samples were stored on the same day they were received at -20 until they were transported on dry ice to Holland. In total there are 3625 dust sock samples (table 2).

Selection of children for dust sample socks:

Austria: All children who had consented to dust sampling, there are 658 dust samples in database.

Germany: *Farm children:* All children who had consented in all parts of the study, and a random sample of children who consented to blood and dust samples. Totally 296/350 (84.6%). *Farm references:* Randomly selected from the children who had given consent, totally 142/150 (94.7%). *Steiner school children:* All of the Steiner school children with a "genuine" anthroposophic lifestyle (as explained above in the blood sampling part), which had consented, 220/368 (59.8%). *Steiner references:* Randomly selected from the children who had given consent, totally 114/170 (67.1%)

Netherlands: All children who gave consent to this part were included, except registered farmers and some people of rural schools whose questionnaires came in at the very end of the questionnaire distribution and recollection part of the project. Totally 579 dust samples.

Sweden: All subjects, who had answered the questionnaire and not already declined add-on investigations when they returned the questionnaire, were invited to the clinical examination and blood sampling. 169 of the children (54 farmers, 49 Steiners, 35 farm children and 31 farm references), had declined all further examinations. Totally 735 dust samples.

Switzerland: In addition to the children already selected for blood sampling an additional random sample of the group who accepted both blood and dust sampling was taken until there were 1000 individuals invited for dust collection, which finally resulted in 842 dust samples.

After exclusion of children not in the right age group (5-13 years) or missing on the group or sex variable there were 3591 children with dust sock samples as shown in table 7.

Table 7. Final number of children used in analyses who have dust sock samples, subdivided by centre and group.

	Farmer		Farmer ref		Steiner		Steiner ref		All groups	
	n	%	n	%	n	%	n	%	n	%
Austria	161	15.5	105	17.1	250	18.3	130	22.9	646	18.0
Germany	347	33.3	129	21.0	208	15.2	117	20.6	801	22.3
Holland	84	8.1	135	22.0	253	18.5	98	17.3	570	15.9
Sweden	232	22.3	85	13.9	309	22.6	109	19.2	735	20.5
Switzerland	217	20.8	159	25.9	348	25.4	113	19.9	837	23.3
All centres (total)	1041	100.0	613	100.0	1368	100.0	567	100.0	3589	100.0

The calculations of the participation rate for the dust socks is shown in table 8, and as can be seen the participation rate differ a lot, as for the blood sampling, between the different countries.

Table 8. Participation rate for the dust socks, subdivided by centre and group.

Country	No. of selected children	Consent for dust socks	Selected for dusts socks	Dust socks	Part.rate (%)
Austria	1589	.	.	658	41,4
Germany	9240	4430	1283	811	30,3
Holland	6403	905	905	579	9,0*
Sweden	1550	954	954	735	47,4
Switzerland	3123	1655	1000	842	44,6
Sum	21905	7944	4142	3625	29,0

* Holland: 150 of the Steiner school children were not asked to participate, for the same reasons discussed for the blood sampling. However this only changes the response rate to 9,3%.

As described above the goal was to analyse 1200 dust socks and we collected 3625. Therefore only a sub sample of the collected socks has been analysed. We selected about 90 children with wheeze and about 90 controls per country from the children of which serum- and sock dust samples were available. Only house dust samples of selected children have been extracted and analyzed (see below), the other samples have been stored. We selected all atopic wheezers (total N=270), a random sample of non-atopic wheezers (N=196) and a random sample of non-atopic controls (N=455). This selection was made irrespective of group (farmer-, Steiner or reference children). We included only one child per family. Children were defined as having wheeze if their parents reported 'wheeze in the past 12 months' or 'wheeze ever'. Children were defined as controls if their parents did not report any of the following symptoms and diseases: 'wheeze ever', 'asthma ever', 'doctor-diagnosed asthma, bronchitis or pseudocroup ever', 'sneezing or a runny or blocked nose without a cold, ever', 'rhinitis ever', 'itchy rash for at least 6 months, ever' and 'eczema ever'. Children were defined as 'atopic' if they were tested PHADIATOP positive at a cut-off IgE level of 0.35 kU/l. Children were defined as 'non-atopic' if they had a negative PHADIATOP test at that cut-off level.

ALK dust collection

The aim was to collect house dust in a subsample of 100 children per country: 50 farm children, 25 Steiner school children and 12 to 13 children of each reference group. Among farmers, only livestock farms with cows and/or pigs were included in this part of the study. The Steiner and reference children were randomly selected from the children whose parents consented to dust sampling, although some country-specific criteria, like proximity of homes to the study centre (the Netherlands), availability of blood sample of index child (Switzerland), and restricted area for

both Steiner and reference children (Austria, Germany and Sweden) were applied. In total, fieldworkers collected dust from the mattresses and living room floors of 271 farm children, 128 Steiner school children and 64 and 67 of their respective references. For farm children, also stable dust was collected. Excluding two of the children who were not in the right age group, resulted in 528 children with an ALK-dust sample as shown in table 9. Some children (n=52), mostly farm children, were excluded from data-analysis, because results of only one child from each family were used.

Table 9. Number of children with ALK-dust samples, subdivided by centre and group.

	Farmer		Farmer ref		Steiner		Steiner ref		All groups	
	n	%	n	%	n	%	n	%	n	%
Austria	50	18.5	13	20.3	25	19.5	11	16.7	99	18.8
Germany	52	19.3	12	18.8	27	21.1	18	27.3	109	20.6
Holland	68	25.2	13	20.3	25	19.5	13	19.7	119	22.5
Sweden	50	18.5	13	20.3	25	19.5	12	18.2	100	18.9
Switzerland	50	18.5	13	20.3	26	20.3	12	18.2	101	19.1
All centres (total)	270	100.0	64	100.0	128	100.0	66	100.0	528	100.0

Dust from mattresses and living room floors was collected on pre-weighed glass fibre filters using vacuum cleaners with sampling nozzles (ALK, Horsholm, Denmark) according to a standardized protocol with photo- and video-instructions. The whole area of the mattress (with under sheets only) was vacuumed for 2 minutes. For living room floors, sampling time and area depended on type of floor covering; carpeted floors, 1 m² for 2 min; smooth floor with ≥ 4 m² rug, 1 m² of rug for 2 min; smooth floor with no rug or smaller rug(s), 2 m² of smooth floor for 4 min. Stable dust was collected at 0.5-1.5 m above the floor from various surfaces (shelves, window sills etc.), using a brush and a dust pan. All samples were shipped in frozen conditions to one laboratory (IRAS, Utrecht, NL) for central analysis.

Analysis of microbial components in the ALK dust sock samples

For house dust samples, filters plus dust were weighed and then extracted in a volume of 5 to 40 ml, determined by the net dust weight (<0.5 g, 5 ml; 0.5 to 1.0 g, 10 ml; 1.0 to 2.0 g, 20 ml; > 2.0 g, 40 ml). Stable dust was sieved through a 0.425 mm mesh and 150 mg of fine dust of each sample was extracted in a volume of 5 ml. Dust from the socks was transferred to preweighed tubes and after weighing it was extracted as for ALK dust samples. Endotoxin, EPS and glucans were extracted sequentially, essentially as described previously (20-22). First, 5 to 40 ml 0.05% (v/v) Tween 20 in pyrogen-free water was added, then suspensions were incubated in an end-over-end roller for 1 h at room temperature, and after centrifugation (15 min, 1,000 x g) 10% of supernatant was harvested and stored at -20°C for endotoxin analysis. The removed supernatant was subsequently replaced with the same volume of PBS solution (10*concentrated) for extraction of EPS. After incubation in an end-over-end roller (1h) and centrifugation (15 min, 2,000 x g), supernatants and remaining dust pellets were stored at -20°C for EPS analysis and glucan extraction respectively. For glucan extraction, each pellet was resuspended in the original volume of PBS-Tween (0.05%), incubated in an end-over-end roller for 15 min, autoclaved for 1 h at 120° and incubated in an end-over-end roller again for 15 min. After centrifugation (15 min, 1,000 x g), supernatants were stored at -20°C until analysis.

Endotoxin was analysed by using a kinetic chromogenic Limulus Amebocyte Lysate (LAL) test, using the same batch for all analyses (BioWhittaker, LAL lysate lot no. 1L676S, LPS standard lot no 2L0090). EPS was analyzed with a specific sandwich EIA for EPS of *Aspergillus* and *Penicillium spp.* The levels of glucans were measured with an inhibition EIA. All concentrations were expressed as endotoxin units, EPS units and micrograms of glucans per gram of dust and per square meter, except for stable samples, for which only levels per gram of dust were calculated. Amounts of dust lower than 0.020 g were considered undetectable (95% of 43 field blank filters had less than 0.0144 g weight increase) and were given a value of 0.013 g. All mattress samples had a detectable amount of dust. For living room (n=25) and stable (n=15) samples with undetectable amounts of dust, no concentrations per gram of dust were calculated, unless the amounts of microbial components were undetectable too. Samples with non-detectable amounts of endotoxin, eps or glucans (< 0.06 EU/ml, <155.3 EPS units/ml, <7.7 µg/ml; n=18, n=14 and n=9 respectively) were given a value of two-thirds of the lowest observed detectable amount per gram of dust or per square meter for the specific component determined. Overall, less than 5% of results are missing, because of the undetectable amounts of dust or because of sampling or extraction failures, or missing mattress areas. The average inter-day/inter-assay coefficients of variation, as determined by testing duplicate aliquots of 10% of all samples on another day as the first aliquot, were 30.5%, 14.5% and 27.4% for endotoxin, EPS and glucans respectively.

Allergen analysis in ALK and dust sock samples

Dust extracts were analysed for Der p 1, Der f 1, Can f I and Fel d 1 (van Strien et al. 2002), using reagents for a sandwich EIA, purchased from Indoor Biotechnologies (Cardiff, UK). The Coefficient of Variation, determined as for the microbial components, ranged from 17.8-27.1%. Concentrations were expressed as nanograms of allergens per gram of dust and per square meter. The lower limits of detection for 5-fold diluted samples were 8 ng/ml for Der p 1, 6 ng/ml for Der f 1, 20 ng/ml for Can f I and 0.4 ng/ml for Fel d 1. The ALK, but not the sock samples, have been analyzed for storage mite allergens as well.

Faecal sampling

To assess the microflora of the gut in relation to certain exposure characteristics, such as use of antibiotics and history of infections as well as dietary habits, including consumption of unpasteurised milk and vegetables preserved by fermentation with lactobacilli, the goal was to obtain faecal samples from 400 children. The same children as for the ALK-samples were invited and 418 samples were obtained, approximately 50% farmers, 25% Steiners and 25% reference children (table 2).

Equipment for the faecal sampling, instructions, and a short questionnaire (including, for example, questions about diet and recent use of antibiotics) were sent to the parents. After sampling, the parents froze the sample in their freezer at roughly -20°C. The sample and the questionnaire were collected by the study personnel when the ALK dust sampling was done. The faecal samples were transported from the study site to the PARSIFAL study centre in the respective country in insulated bags with normal ice. The samples were then stored at -20°C until the collection of all the faecal samples was finished. Then all the samples were transported to the Swedish study centre and moved to a -80° C freezer.

Four of the children were not in the right age range (5-13 years), so finally 414 children with a faecal sample were included as shown in table 10.

Table 10. Number of children with faecal samples, subdivided by centre and group.

	Farmer		Farmer ref		Steiner		Steiner ref		All groups	
	N	%	n	%	n	%	n	%	n	%
Austria	46	21.6	12	25.0	24	22.9	12	25.0	94	22.7
Germany	47	22.1	10	20.8	20	19.0	10	20.8	87	21.0
Holland	49	23.0	6	12.5	19	18.1	10	20.8	84	20.3
Sweden	38	17.8	11	22.9	21	20.0	8	16.7	78	18.8
Switzerland	33	15.5	9	18.8	21	20.0	8	16.7	71	17.1
All centres (total)	213	100.0	48	100.0	105	100.0	48	100.0	414	100.0

Different methods for extraction of DNA from bacteria in the faecal samples have been tested. It was decided to use the Qiagen kit (QiAmp DNA extraction kit) and MoBio kit (Ultra clean Soil DNA kit, MoBio). The analyses use the T-RFLP method and include determination of a variety of bacteria based on genotyping of DNA: Lactobacilli, Spirochetes, Mycobacteria and *Lawsonia intracellulare*. The analyses are performed in co-operation with the Swedish University of Agricultural Sciences and the Swedish Institute for Infectious Disease Control.

Bronchial hyperresponsiveness (BHR) test

It was planned to perform 100 BHR tests in the wheezer group and 100 BHR tests in the non-wheezer group, evenly distributed in the different subgroups in each country. The wheezers were selected from those who had a positive answer to question B4 (wheeze during the last 12 months) in the main questionnaire, and the non-wheezers who had answered no. Eventually 893 BHR-tests were performed, 366 in wheezers and 527 in non-wheezers (table 2).

The BHR tests were performed in clinics or at the children's schools, according to a common study protocol. A minimum of two baseline spiromograms were recorded and the highest of two reproducible (within 5%) measures of FEV1 was recorded as baseline FEV1. Bronchial reactivity was then assessed by changes in FEV1 after inhalation of nebulised saline (4.5% hypertonic saline inhalation) using ultrasound nebulisers (DeVilbiss Sunrise Medical, Langen, Germany). All children were asked to withhold all asthma medications for at least 12 hours before the BHR-test. In children with a baseline FEV1 of less than 75% predicted, no bronchial challenge was performed and an inhaled bronchodilator (salbutamol) was administered.

In one study centre (Sweden) the nose clip was not used when inhaling the salt aerosol, but the children were instructed not to inhale through the nose. Some of the children thought that the salt aerosol tasted badly and reduced mouth inhalation. This was noted when the nebuliser chamber plus the aerosol tube were weighed after the final challenge and the total amount of nebulised saline was calculated.

Eight of the children were not in the right age range, so finally 885 children with a BHR test were included as shown in table 11.

Table 11. Number of children who completed BHR tests, subdivided by centre and group.

		Farmer		Farmer co		Steiner		Steiner co		All groups	
		n	%	n	%	n	%	n	%	n	%
Austria	Wheezer										
	No	21	10.0	31	16.4	28	10.1	25	12.0	105	11.9
	Yes	14	6.6	1	0.5	23	8.3	15	7.2	53	6.0
	Total	35	16.6	32	16.9	51	18.4	40	19.2	158	17.9
Germany	Wheezer										
	No	31	14.7	26	13.8	33	11.9	27	13.0	117	13.2
	Yes	20	9.5	25	13.2	21	7.6	24	11.5	90	10.2
	Total	51	24.2	51	27.0	54	19.5	51	24.5	207	23.4
Holland	Wheezer										
	No	19	9.0	19	10.1	30	10.8	23	11.1	91	10.3
	Yes	17	8.1	19	10.1	18	6.5	21	10.1	75	8.5
	Total	36	17.1	38	20.1	48	17.3	44	21.2	166	18.8
Sweden	Wheezer										
	No	28	13.3	17	9.0	31	11.2	18	8.7	94	10.6
	Yes	16	7.6	5	2.6	30	10.8	12	5.8	63	7.1
	Total	44	20.9	22	11.6	61	22.0	30	14.4	157	17.7
Switzerland	Wheezer										
	No	29	13.7	26	13.8	36	13.0	27	13.0	118	13.3
	Yes	16	7.6	20	10.6	27	9.7	16	7.7	79	8.9
	Total	45	21.3	46	24.3	63	22.7	43	20.7	197	22.3
All centres	Wheezer										
	No	128	60.7	119	63.0	158	57.0	120	57.7	525	59.3
	Yes	83	39.3	70	37.0	119	43.0	88	42.3	360	40.7
	Total	211	100	189	100	277	100	208	100	885	100

24-h-Diet Recall

The goal of the 24-hour dietary recall was to evaluate whether the foods reported in the main questionnaire were actually consumed by the children. A random sample of 25 children per subgroup, who had not declined participation, was selected in each country aiming at a total of about 500 children. If parents could not be contacted following several attempts, new children of the same subgroup were selected to reach the required number of 100 interviews per centre. The 24-hour recall was administered by trained interviewers on average 17.4 months (range: means Austria: 13.2 months; Holland: 25.8 months) after the main questionnaire. The selected participants were contacted and if they agreed to participate, the interviewer would make an appointment to call them during the following week asking both the parent and the index child to be present for the phone interview. During the interview, the interviewer asked the parent together with the child to recall all food items and beverages consumed during the previous 24 hours. The interviewer also inquired whether the prior 24 hours represented a typical day with respect to the dietary habits of the child. The 24-hour recall always pertained to diet on a weekday. After the interview was completed, the interviewer entered the information into a database that was restricted to the foods of interest from the main questionnaire. Thus the database established for the 24-hour recall correlated directly with the database from the dietary questionnaire.

Finally a total of 493 children were included in the 24-h diet recall component of this study. The sample included 255 girls and 237 boys, and the mean age was 8.8 years. Of the sample, 124 were farmers' children, 122 children from Rudolf Steiner schools, 122 farmer reference children, and 125 Rudolf Steiner School reference children.

Ethical permission

Ethical committees in the respective country have approved all parts of the project. The ethical committee at the Karolinska Institute approved the main application for the project on May 31, 2000 (00-140).

6.4 Scientific achievements

As shown in table 1, altogether 15137 questionnaires were collected, 1282 in Austria, 6963 in Germany, 3230 in Holland, 1109 in Sweden and 2553 in Switzerland. Of these 14893 study subjects were in the right age and were not missing on sex or group as can be seen in table 3. The results presented below starts with descriptive background data from the questionnaire, subdivided by group. After that results from the different examinations are presented and finally more detailed comparisons between the farm group and their reference group and the Steiner school group and their reference group are presented. The analyses below are only subdivided by group, however, most of the analyses can also be found subdivided by country in appendix 2. It should be stressed that further in depth analyses of the PARSIFAL material are ongoing and many of the results presented in this report should be viewed upon as preliminary, particularly in relation to interpretation and conclusions.

Background characteristics

In table 12 the demographics for the study population divided by group are shown. As can be seen the study population consists of 19% farm children, 37% farm references, 31% Steiner school children and 14% Steiner references. The Steiner school and farm children are slightly older than their respective references (9.0 and 9.1 years compared to 8.7 and 8.8 years for their reference groups). There are slightly more boys than girls in the farm and farm reference groups whereas girls constitute just over 50% in the Steiner school and Steiner reference groups. University education of the parents is much more common in the Steiner school group (65%) than in the Steiner reference group (38%), the farm group (11%) and the farm reference group (18%). Only about 1% of the farm children and farm reference children were born outside their respective countries compared to 6% in the Steiner group and 4,5% in the Steiner reference group.

Table 12. Demographics for the study population, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Age group										
	5-7 years	736	26.1	1614	29.7	1083	23.5	553	27.3	3986	26.8
	8-10 years	1375	48.7	2886	53.1	2324	50.5	1106	54.6	7691	51.6
	11-13 years	712	25.2	940	17.3	1199	26.0	365	18.0	3216	21.6
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Sex										
	Boy	1453	51.5	2796	51.4	2209	48.0	995	49.2	7453	50.0
	Girl	1370	48.5	2644	48.6	2397	52.0	1029	50.8	7440	50.0
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Parent's education										
	Elementary school or lower	962	34.1	1578	29.0	108	2.3	261	12.9	2909	19.5
	Gymnasium	1468	52.0	2748	50.5	1401	30.4	925	45.7	6542	43.9
	University education	303	10.7	965	17.7	2985	64.8	766	37.8	5019	33.7
	Not reporting	90	3.2	149	2.7	112	2.4	72	3.6	423	2.8
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Born outside country										
	No	2798	99.1	5343	98.2	4323	93.9	1928	95.3	14392	96.6
Yes	21	0.7	89	1.6	277	6.0	92	4.5	479	3.2	
Not reporting	4	0.1	8	0.1	6	0.1	4	0.2	22	0.1	
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

In table 13 the number of older and younger siblings in the respective groups is presented. The children in both the Farmer (1.9 older and 1.6 younger siblings) and Steiner groups (1.8 older and 1.5 younger siblings) have slightly more siblings, both older and younger than their respective reference groups (1.5 older and 1.3 younger siblings in both reference groups).

Table 13. Number of older/younger siblings, subdivided by group.

			Farmer	Farmer ref	Steiner	Steiner ref	All groups
All centres	Number of older brothers and sisters	Mean	1.3	0.8	1.1	0.8	1.0
		N	2751	5264	4474	1971	14460
		Not reporting	72	176	132	53	433
	Number of younger brothers and sisters	Mean	1.5	1.1	1.2	1.0	1.2
		N	1776	3326	2830	1194	9126
		Not reporting	1047	2114	1776	830	5767
Total	N	2823	5440	4606	2024	14893	

In table 14 the mean age, weight and height are presented. It can be noted that the Farm and Steiner school children are slightly taller than the reference groups and that the Farm children weigh more than the other children. These two groups are, however, also slightly older on average.

Table 14. Mean age, weight and height, subdivided by group.

			Farmer	Farmer ref	Steiner	Steiner ref	All groups
All centres	Age	Mean	9.0	8.7	9.1	8.8	8.9
		Std	1.9	1.8	1.9	1.8	1.8
		Median	9.0	9.0	9.0	9.0	9.0
		Min	5.0	5.0	5.0	5.0	5.0
		Max	13.0	13.0	13.0	13.0	13.0
		n	2823	5440	4606	2024	14893
		Not reporting	0	0	0	0	0
	Weight [Kg]	Mean	34.2	32.0	32.2	32.5	32.5
		Std	10.0	9.0	8.5	8.9	9.1
		Median	33.0	30.0	31.0	31.0	31.0
		Min	15.0	12.0	13.0	14.0	12.0
		Max	82.0	85.0	89.0	73.0	89.0
		n	2538	4839	4056	1804	13237
		Not reporting	285	601	550	220	1656
	Height [Cm]	Mean	139.3	137.1	139.2	137.7	138.3
		Std	12.4	11.7	12.2	11.8	12.1
		Median	140.0	137.0	140.0	138.0	138.0
		Min	95.0	99.0	100.0	100.0	95.0
		Max	183.0	179.0	182.0	177.0	183.0
		n	2513	4741	4026	1748	13028
		Not reporting	310	699	580	276	1865
Total	N	2823	5440	4606	2024	14893	

In table 15 the data regarding breast feeding are presented. As shown the Steiner children were breastfed exclusively during a longer period than the other children (51% during 5 months or more compared to 23% in the other groups).

Table 15. Percentage of children who were breastfed, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Breast feed exclusively ≥ 5 months, QE5										
	No breastfeeding	607	21.5	1493	27.4	165	3.6	358	17.7	2623	17.6
	Breastfeeding 0-4 months	1505	53.3	2542	46.7	1913	41.5	1001	49.5	6961	46.7
	Breastfeeding ≥ 5 months	607	21.5	1195	22.0	2357	51.2	527	26.0	4686	31.5
	Not reporting	104	3.7	210	3.9	171	3.7	138	6.8	623	4.2
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

In table 16 the prevalence of parental atopy (defined as parental asthma and/or hay fever) and the prevalence of parental asthma, parental atopic dermatitis and parental hay fever is also presented. Parental atopy is more common in the Steiner and Steiner reference group (44% and 40%) than in the farmer reference group (30%) and is even lower in the farmer group (20%).

Table 16. Parental atopy, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
	Parental atopy**										
All centres	Yes	553	19.6	1646	30.3	2033	44.1	814	40.2	5046	33.9
	No	2247	79.6	3763	69.2	2534	55.0	1188	58.7	9732	65.3
	Not reporting	23	0.8	31	0.6	39	0.8	22	1.1	115	0.8
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Parental asthma										
	Yes	270	9.6	549	10.1	745	16.2	270	13.3	1834	12.3
	No	2467	87.4	4650	85.5	3534	76.7	1635	80.8	12286	82.5
	Not reporting	86	3.0	241	4.4	327	7.1	119	5.9	773	5.2
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Parental hay fever										
	Yes	369	13.1	1395	25.6	1821	39.5	725	35.8	4310	28.9
	No	2343	83.0	3855	70.9	2551	55.4	1210	59.8	9959	66.9
	Not reporting	111	3.9	190	3.5	234	5.1	89	4.4	624	4.2
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Parental atopic dermatitis										
	Yes	448	15.9	942	17.3	1228	26.7	424	20.9	3042	20.4
	No	2272	80.5	4248	78.1	3048	66.2	1473	72.8	11041	74.1
	Not reporting	103	3.6	250	4.6	330	7.2	127	6.3	810	5.4
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

In table 17 the prevalence of maternal smoking during pregnancy and during the first year of child's life and in table 18 the prevalence of current environmental smoking at age of interview are presented. Maternal smoking during pregnancy was more common in the reference groups (13%) than in the Farm (6%) and Steiner school group (8%), similarly smoking during first year of life was more common in the reference groups (20%) than in the Farm (9%) and Steiner school group (12%). The same trend is seen for current environmental smoking, where 25% of the children in the reference groups live in an environment with environmental smoking, whereas the corresponding figure for the children to farmers is 15% and 14% for the Steiner school children. Holland and Sweden have the highest prevalence of maternal smoking during pregnancy, 16% and 13%, compared to the other countries, ca 8%. In Holland smoking during first year of life was more common (21%) than in the other countries (14%), (see appendix 2).

Table 17. Maternal smoking during pregnancy and during first year of life, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Maternal smoking in pregnancy										
	Yes	180	6.4	668	12.3	366	7.9	277	13.7	1491	10.0
	No	2601	92.1	4700	86.4	4165	90.4	1709	84.4	13175	88.5
	Not reporting	42	1.5	72	1.3	75	1.6	38	1.9	227	1.5
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Maternal smoking in first year of child's life										
	Yes	266	9.4	1072	19.7	568	12.3	390	19.3	2296	15.4
	No	2454	86.9	4225	77.7	3889	84.4	1561	77.1	12129	81.4
	Not reporting	103	3.6	143	2.6	149	3.2	73	3.6	468	3.1
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

Table 18. Current environmental smoking, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Current Environmental Tobacco Smoke										
	No	2362	83.7	4052	74.5	3931	85.3	1445	71.4	11790	79.2
	Yes	435	15.4	1326	24.4	630	13.7	545	26.9	2936	19.7
	Not reporting	26	0.9	62	1.1	45	1.0	34	1.7	167	1.1
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Number of cigarettes/day currently										
	No cigarettes	2362	83.7	4052	74.5	3931	85.3	1445	71.4	11790	79.2
	Less than 10 cigarettes/day	167	5.9	380	7.0	244	5.3	149	7.4	940	6.3
	10-20 cigarettes/day	162	5.7	576	10.6	258	5.6	235	11.6	1231	8.3
	more than 20 cigarettes/day	54	1.9	266	4.9	81	1.8	101	5.0	502	3.4
	Not reporting	78	2.8	166	3.1	92	2.0	94	4.6	430	2.9
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

In table 19 the prevalence of household pets during pregnancy, the first year of life and current household pets are shown. As can be seen the Farm and the Steiner families had more household pets during pregnancy, during the first year of life and also have a higher prevalence of household pets at the time of answering the questionnaire.

Table 19. Household pets during pregnancy, first year of life and current house hold pets, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Household pets during pregnancy										
	No	1544	54.7	3714	68.3	3014	65.4	1467	72.5	9739	65.4
	Yes	1213	43.0	1675	30.8	1563	33.9	530	26.2	4981	33.4
	Not reporting	66	2.3	51	0.9	29	0.6	27	1.3	173	1.2
	Total	2823	100	5440	100	4606	100	2024	100	14893	100
	Household pets during the first year of life										
	No	1500	53.1	3602	66.2	2890	62.7	1426	70.5	9418	63.2
	Yes	1257	44.5	1787	32.8	1687	36.6	571	28.2	5302	35.6
	Not reporting	66	2.3	51	0.9	29	0.6	27	1.3	173	1.2
	Total	2823	100	5440	100	4606	100	2024	100	14893	100
	Household pets now										
	No	1111	39.4	2384	43.8	1724	37.4	984	48.6	6203	41.7
	Yes	1646	58.3	3005	55.2	2853	61.9	1013	50.0	8517	57.2
	Not reporting	66	2.3	51	0.9	29	0.6	27	1.3	173	1.2
	Total	2823	100	5440	100	4606	100	2024	100	14893	100

Selected life style variables

In table 20 the use of antibiotics for the different groups is described. The Steiner school children start using antibiotics at a later age than the other groups (only 17% of the Steiner school children had used antibiotics during the first year of life compared to approximately 33% in the other groups), and a larger proportion of the Steiner school children compared to the other groups have never used antibiotics (42% compared to 15%).

Table 20. Use of antibiotics, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
	Total										
All centres	Never use	442	15.7	785	14.4	1914	41.6	305	15.1	3446	23.1
	1st use >12 mth	1206	42.7	2548	46.8	1750	38.0	974	48.1	6478	43.5
	1st use 0-12 mth	978	34.6	1740	32.0	788	17.1	623	30.8	4129	27.7
	Not reporting	197	7.0	367	6.7	154	3.3	122	6.0	840	5.6

In table 21 the use of antipyretics for the different groups is described. The trend is the same as for antibiotics. The Steiner school children start using antipyretics at a later age than the other groups (only 20% of the Steiner school children had used antipyretics during the first year of life compared to approximately 54% in the other groups), and a larger proportion of the Steiner school children compared to the other groups have never used antipyretics (43% compared to 9%).

Table 21. Use of antipyretics, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
	Total										
All centres	Never use	306	10.8	454	8.3	1970	42.8	167	8.3	2897	19.5
	1st use >12 mth	782	27.7	1679	30.9	1539	33.4	646	31.9	4646	31.2
	1st use 0-12 mth	1523	53.9	3016	55.4	923	20.0	1072	53.0	6534	43.9
	Not reporting	212	7.5	291	5.3	174	3.8	139	6.9	816	5.5

In tables 22 to 30 the prevalence of vaccination against measles, mumps, whooping cough, hemophilus influenza, diphtheria, tetanus, polio and tuberculosis as well as the numbers of children who have had the different diseases are presented. The Steiner group has a much lower prevalence of vaccination against measles, mumps, whooping cough and hemophilus influenza. The Steiner children also have a lower prevalence of vaccination against polio and tuberculosis and a slightly lower prevalence of vaccination against diphtheria. The only vaccination where there is no difference is Tetanus. The percentage of children who have had measles, mumps, whooping cough and hemophilus influenza is much higher among the Steiner children than among the other groups. For the other diseases studied in table 22 to 29 there are no such differences.

Table 22. Prevalence of vaccination against measles and numbers of children who have had measles, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Child vaccination: Measles										
	No	259	9.2	506	9.3	2642	57.4	159	7.9	3566	23.9
	Yes	2322	82.3	4487	82.5	1448	31.4	1648	81.4	9905	66.5
	Don't know	33	1.2	67	1.2	43	0.9	37	1.8	180	1.2
	Not reporting	209	7.4	380	7.0	473	10.3	180	8.9	1242	8.3
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Child had Measles										
	No	2142	75.9	4282	78.7	2689	58.4	1618	79.9	10731	72.1
	Yes	445	15.8	705	13.0	1536	33.3	208	10.3	2894	19.4
	Don't know	68	2.4	107	2.0	112	2.4	33	1.6	320	2.1
	Not reporting	168	6.0	346	6.4	269	5.8	165	8.2	948	6.4
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

Table 23. Prevalence of vaccination against rubella and numbers of children who have had rubella, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Child vaccination: Rubella										
	No	365	12.9	647	11.9	2794	60.7	258	12.7	4064	27.3
	Yes	2093	74.1	4240	77.9	1223	26.6	1497	74.0	9053	60.8
	Don't know	58	2.1	87	1.6	54	1.2	49	2.4	248	1.7
	Not reporting	307	10.9	466	8.6	535	11.6	220	10.9	1528	10.3
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Child had Rubella										
	No	2297	81.4	4547	83.6	2606	56.6	1626	80.3	11076	74.4
	Yes	211	7.5	422	7.8	1245	27.0	174	8.6	2052	13.8
	Don't know	91	3.2	118	2.2	421	9.1	52	2.6	682	4.6
	Not reporting	224	7.9	353	6.5	334	7.3	172	8.5	1083	7.3
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

Table 24. Prevalence of vaccination against mumps and numbers of children who have had mumps, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Child vaccination: Mumps										
	No	269	9.5	514	9.4	2684	58.3	183	9.0	3650	24.5
	Yes	2235	79.2	4420	81.3	1361	29.5	1605	79.3	9621	64.6
	Don't know	44	1.6	77	1.4	49	1.1	42	2.1	212	1.4
	Not reporting	275	9.7	429	7.9	512	11.1	194	9.6	1410	9.5
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Child had Mumps										
	No	2331	82.6	4695	86.3	2691	58.4	1671	82.6	11388	76.5
	Yes	234	8.3	284	5.2	1368	29.7	147	7.3	2033	13.7
	Don't know	50	1.8	68	1.3	196	4.3	31	1.5	345	2.3
	Not reporting	208	7.4	393	7.2	351	7.6	175	8.6	1127	7.6
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

Table 25. Prevalence of vaccination against whooping cough and numbers of children who have had whooping cough, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Child vaccination: Whooping cough										
	No	565	20.0	1163	21.4	2602	56.5	411	20.3	4741	31.8
	Yes	1745	61.8	3323	61.1	1342	29.1	1230	60.8	7640	51.3
	Don't know	102	3.6	172	3.2	65	1.4	92	4.5	431	2.9
	Not reporting	411	14.6	782	14.4	597	13.0	291	14.4	2081	14.0
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Child had Whooping cough										
	No	2243	79.5	4286	78.8	2604	56.5	1585	78.3	10718	72.0
	Yes	341	12.1	731	13.4	1545	33.5	227	11.2	2844	19.1
	Don't know	43	1.5	99	1.8	188	4.1	47	2.3	377	2.5
	Not reporting	196	6.9	324	6.0	269	5.8	165	8.2	954	6.4
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

Table 26. Prevalence of vaccination against hemophilus influenza and numbers of children who have had hemophilus influenza, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
	Child vaccination: Hemophilus Influenza										
All centres	No	621	22.0	1003	18.4	2495	54.2	343	16.9	4462	30.0
	Yes	1289	45.7	3048	56.0	1121	24.3	1092	54.0	6550	44.0
	Don't know	205	7.3	250	4.6	141	3.1	131	6.5	727	4.9
	Not reporting	708	25.1	1139	20.9	849	18.4	458	22.6	3154	21.2
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Child had Hemophilus Influenza										
	No	2006	71.1	3777	69.4	3116	67.7	1400	69.2	10299	69.2
	Yes	11	0.4	22	0.4	38	0.8	14	0.7	85	0.6
	Don't know	74	2.6	78	1.4	111	2.4	33	1.6	296	2.0
	Not reporting	732	25.9	1563	28.7	1341	29.1	577	28.5	4213	28.3
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

Table 27. Prevalence of vaccination against diphtheria and numbers of children who have had diphtheria, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
	Child vaccination: Diphtheria										
All centres	No	51	1.8	84	1.5	596	12.9	51	2.5	782	5.3
	Yes	2493	88.3	4902	90.1	3735	81.1	1741	86.0	12871	86.4
	Don't know	52	1.8	65	1.2	48	1.0	37	1.8	202	1.4
	Not reporting	227	8.0	389	7.2	227	4.9	195	9.6	1038	7.0
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Child had Diphtheria										
	No	2552	90.4	4994	91.8	4118	89.4	1814	89.6	13478	90.5
	Yes	4	0.1	12	0.2	9	0.2	5	0.2	30	0.2
	Don't know	13	0.5	11	0.2	28	0.6	8	0.4	60	0.4
	Not reporting	254	9.0	423	7.8	451	9.8	197	9.7	1325	8.9
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

Table 28. Prevalence of vaccination against tetanus and numbers of children who have had tetanus, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Child vaccination: Tetanus										
	No	47	1.7	69	1.3	294	6.4	33	1.6	443	3.0
	Yes	2545	90.2	4953	91.0	4116	89.4	1776	87.7	13390	89.9
	Don't know	27	1.0	47	0.9	27	0.6	28	1.4	129	0.9
	Not reporting	204	7.2	371	6.8	169	3.7	187	9.2	931	6.3
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Child had Tetanus										
	No	2557	90.6	4995	91.8	4121	89.5	1816	89.7	13489	90.6
	Yes	7	0.2	10	0.2	10	0.2	4	0.2	31	0.2
	Don't know	8	0.3	12	0.2	26	0.6	9	0.4	55	0.4
	Not reporting	251	8.9	423	7.8	449	9.7	195	9.6	1318	8.8
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

Table 29. Prevalence of vaccination against polio and numbers of children who have had polio, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Child vaccination: Polio										
	No	28	1.0	70	1.3	548	11.9	33	1.6	679	4.6
	Yes	2594	91.9	5031	92.5	3822	83.0	1805	89.2	13252	89.0
	Don't know	23	0.8	32	0.6	36	0.8	24	1.2	115	0.8
	Not reporting	178	6.3	307	5.6	200	4.3	162	8.0	847	5.7
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Child had Polio										
	No	2557	90.6	4998	91.9	4119	89.4	1816	89.7	13490	90.6
	Yes	4	0.1	10	0.2	10	0.2	5	0.2	29	0.2
	Don't know	8	0.3	9	0.2	28	0.6	8	0.4	53	0.4
	Not reporting	254	9.0	423	7.8	449	9.7	195	9.6	1321	8.9
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

Table 30. Prevalence of vaccination with BCG and numbers of children who have had tuberculosis, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Child vaccination: BCG										
	No	993	35.2	2170	39.9	2787	60.5	734	36.3	6684	44.9
	Yes	719	25.5	1113	20.5	593	12.9	562	27.8	2987	20.1
	Don't know	196	6.9	291	5.3	160	3.5	105	5.2	752	5.0
	Not reporting	915	32.4	1866	34.3	1066	23.1	623	30.8	4470	30.0
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Child had Tuberculosis										
	No	2547	90.2	4991	91.7	4110	89.2	1820	89.9	13468	90.4
	Yes	6	0.2	8	0.1	8	0.2	2	0.1	24	0.2
	Don't know	8	0.3	10	0.2	27	0.6	8	0.4	53	0.4
	Not reporting	262	9.3	431	7.9	461	10.0	194	9.6	1348	9.1
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

Table 31 shows the use of organic and biodynamic food. As can be seen the Steiner school group uses organic and biodynamic food to a much larger extent than the other groups: Steiner school children 77%, Steiner references 20% and Farm and Farm references 14%. The trend is the same for all countries, but the differences are greater in Germany than in the other countries (see appendix 2).

Table 31. Use of organic and biodynamic food, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Total										
	Conventional	2266	80.3	4305	79.1	1022	22.2	1468	72.5	9061	60.8
	Org/Biodynamic	415	14.7	746	13.7	3522	76.5	400	19.8	5083	34.1
	Not reporting	142	5.0	389	7.2	62	1.3	156	7.7	749	5.0

In table 32 the prevalence of children whose mothers were in contact with farm animals during pregnancy, as well as the prevalence of children who were in contact with animals during the first or second year of life. Not surprisingly, children to farmers were in contact with farm animals during both their first and second year of life to a much larger extent than the other groups and also the mothers to the farm children were in contact with farm animals during the pregnancy to a much larger extent than the other mothers (84% among farmers compared to approximately 26% among farm references and Steiner school children and only 17% among Steiner references).

Table 32. Prevalence of children whose mothers were in contact with farm animals during pregnancy, the prevalence of children who were in contact with animals during the first year of life and during the second year of life, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Mothers contact with farm animals during pregnancy										
	No	414	14.7	3811	70.1	3264	70.9	1598	79.0	9087	61.0
	Yes	2369	83.9	1438	26.4	1142	24.8	341	16.8	5290	35.5
	Not reporting	40	1.4	191	3.5	200	4.3	85	4.2	516	3.5
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Contact with farm animals during the first year of life										
	No	964	34.1	1992	36.6	1780	38.6	567	28.0	5303	35.6
	Yes	1703	60.3	854	15.7	822	17.8	208	10.3	3587	24.1
	Not reporting	156	5.5	2594	47.7	2004	43.5	1249	61.7	6003	40.3
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Contact with farm animals during age 13-24 months										
	No	778	27.6	1868	34.3	1749	38.0	597	29.5	4992	33.5
	Yes	1889	66.9	978	18.0	853	18.5	178	8.8	3898	26.2
	Not reporting	156	5.5	2594	47.7	2004	43.5	1249	61.7	6003	40.3
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

In table 33 the prevalence of children who live on a farm and the prevalence of parents to the children who run a farm is presented. Almost 100% of the farmer children live on a farm and 95% of their parents run a farm, compared to the reference groups were almost none of the children live on a farm. In the Steiner group 4% of the children live on a farm and 3% of their parents run a farm.

Table 33. Prevalence of children who live on farm, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Child lives on a farm										
	No	9	0.3	5400	99.3	4398	95.5	1996	98.6	11803	79.3
	Yes	2806	99.4	29	0.5	190	4.1	15	0.7	3040	20.4
	3	2	0.1	0	0	1	0.0	0	0	3	0.0
	Not reporting	6	0.2	11	0.2	17	0.4	13	0.6	47	0.3
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Running a farm										
	No	80	2.8	230	4.2	114	2.5	82	4.1	506	3.4
	Yes	2660	94.2	52	1.0	141	3.1	4	0.2	2857	19.2
	Not reporting	83	2.9	5158	94.8	4351	94.5	1938	95.8	11530	77.4
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

In table 34 the extent of farming for the farmers are described and as can be seen a majority of the farmer families are full time farmers.

Table 34. Extent of farming for the farmers, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
	Extent of farming current										
All centres	Full time farming current	1689	59.8	22	0.4	57	1.2	3	0.1	1771	11.9
	Part time farming current	745	26.4	20	0.4	38	0.8	0	0	803	5.4
	Farming for own needs current	225	8.0	10	0.2	46	1.0	1	0.0	282	1.9
	Not reporting	164	5.8	5388	99.0	4465	96.9	2020	99.8	12037	80.8
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

Health outcomes and sensitisation

In table 35 the prevalence of asthma, wheezing and coughing at night for the different centres and groups are described. The prevalence of doctor's diagnosis of asthma is lower in the farm group (6%) compared to the farm references (9%) and appears also to be lower in the Steiner school group (9%) compared to the Steiner references (11%). For current wheezing (during the last 12 months) the prevalence again tends to be lower among the farm children than among the farmer references (4% compared to 6%). However, it is higher in the Steiner school group than in the Steiner reference group (9% compared to 5%).

Table 35. Prevalence of asthma, wheezing and coughing at night, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Doctor's diagnosis of asthma										
	Yes	172	6.1	484	8.9	421	9.1	217	10.7	1294	8.7
	No	2578	91.3	4846	89.1	4090	88.8	1762	87.1	13276	89.1
	Not reporting	73	2.6	110	2.0	95	2.1	45	2.2	323	2.2
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Current wheezing + doctor's diagnosis of asthma										
	Positive	75	2.7	243	4.5	224	4.9	108	5.3	650	4.4
	Intermediate	248	8.8	557	10.2	502	10.9	228	11.3	1535	10.3
	Negative	2496	88.4	4631	85.1	3876	84.2	1688	83.4	12691	85.2
	Not reporting	4	0.1	9	0.2	4	0.1	0	0	17	0.1
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Current wheezing (12 mo.)										
	Yes	140	5.0	412	7.6	398	8.6	168	8.3	1118	7.5
	No	2655	94.0	4962	91.2	4150	90.1	1834	90.6	13601	91.3
	Not reporting	28	1.0	66	1.2	58	1.3	22	1.1	174	1.2
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Coughing at night (12 Mo.)										
	Yes	211	7.5	672	12.4	527	11.4	293	14.5	1703	11.4
	No	2591	91.8	4726	86.9	4045	87.8	1719	84.9	13081	87.8
	Not reporting	21	0.7	42	0.8	34	0.7	12	0.6	109	0.7
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

In table 36 the prevalence of current atopic dermatitis, atopic dermatitis at specific locations and atopic dermatitis during the last 12 months for the different centres and groups are described. The prevalence of current atopic dermatitis in the farm group appears lower than in the farm reference group (5% compared to 8%) and lower in the Steiner school group than in the Steiner reference group (8% compared to 10%).

Table 36. Prevalence of, atopic dermatitis, atopic dermatitis at specific locations and atopic dermatitis during the last 12 months, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Current atopic dermatitis										
	Yes	159	5.6	416	7.6	392	8.5	211	10.4	1178	7.9
	No	2647	93.8	4999	91.9	4179	90.7	1805	89.2	13630	91.5
	Not reporting	17	0.6	25	0.5	35	0.8	8	0.4	85	0.6
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Atopic dermatitis symptoms ever + specific location										
	Yes	277	9.8	618	11.4	645	14.0	320	15.8	1860	12.5
	No	2519	89.2	4776	87.8	3901	84.7	1688	83.4	12884	86.5
	Not reporting	27	1.0	46	0.8	60	1.3	16	0.8	149	1.0
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Atopic dermatitis symptoms (12 mo.)										
	Yes	239	8.5	526	9.7	525	11.4	294	14.5	1584	10.6
	No	2552	90.4	4873	89.6	4015	87.2	1717	84.8	13157	88.3
	Not reporting	32	1.1	41	0.8	66	1.4	13	0.6	152	1.0
Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0	

In table 37 the prevalence is described of hay fever symptoms during the last 12 months and doctor's diagnosis of hay fever for the different centres and groups. The prevalence of hay fever symptoms is lower in the farm group than in the farm reference group (3% compared to 8%) and lower in the Steiner school group than in the Steiner reference group (8% compared to 11%).

Table 37. Prevalence of hay fever symptoms during the last 12 months and doctor diagnosis of hay fever, subdivided by group.

		Farm		Farm ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Hay fever symptoms (12 mo.)										
	Yes	91	3.2	416	7.6	363	7.9	212	10.5	1082	7.3
	No	2710	96.0	4957	91.1	4195	91.1	1786	88.2	13648	91.6
	Not reporting	22	0.8	67	1.2	48	1.0	26	1.3	163	1.1
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0
	Doctor's diagnosis of hay fever										
	Yes	48	1.7	295	5.4	244	5.3	147	7.3	734	4.9
	No	2735	96.9	5052	92.9	4264	92.6	1828	90.3	13879	93.2
	Not reporting	40	1.4	93	1.7	98	2.1	49	2.4	280	1.9
	Total	2823	100.0	5440	100.0	4606	100.0	2024	100.0	14893	100.0

In table 38 the results from the Phadiatop (IgE against common inhalant allergens)-analyses are described. As shown the farm group has a lower prevalence (20%) than the farm reference group (32%) and the Steiner school group has a lower prevalence (31%) than the Steiner reference group (36%).

Table 38. Results from Phadiatop-analyses, subdivided by group. (unit kU/L)

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Phadiatop										
	Neg	1101	79.4	475	67.8	829	69.0	407	64.2	2812	71.7
	Pos	285	20.6	226	32.2	372	31.0	227	35.8	1110	28.3
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	RAST class										
	less than 0.35	1101	79.4	475	67.8	829	69.0	407	64.2	2812	71.7
	0.35 - <0.7	34	2.5	39	5.6	59	4.9	24	3.8	156	4.0
	0.7 - <3.5	77	5.6	37	5.3	99	8.2	49	7.7	262	6.7
	3.5 - <17.5	33	2.4	48	6.8	74	6.2	49	7.7	204	5.2
	17.5 - <50	35	2.5	57	8.1	77	6.4	43	6.8	212	5.4
	50 - <100	17	1.2	27	3.9	51	4.2	43	6.8	138	3.5
	100 or more	4	0.3	10	1.4	10	0.8	12	1.9	36	0.9
	Missing	85	6.1	8	1.1	2	0.2	7	1.1	102	2.6
Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0	

In table 39 the results from the combined IgE-analyses (value above the cut-off-point for at least one of the IgE-analyses (Phadiatop, Fx-5, grass, tree, horse, cat, *Dermatophagoides pteronyssinus* and *Lepidoglyphus destructor*) are shown with two different cut-off points used to classify atopy (0.35 kU/L and 3.5 kU/L). The farm children have a lower prevalence (23%) than the farm references (35%) and the Steiner school children a lower prevalence (32%) than the Steiner references (39%).

Table 39. Prevalence of atopy using 0.35 kU/l and 3.5 kU/l as cut-off points, subdivided by group.

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Atopic sensitization, cut of 0.35 kU/L										
	No	1072	77.3	458	65.3	814	67.8	386	60.9	2730	69.6
	Yes	314	22.7	243	34.7	387	32.2	248	39.1	1192	30.4
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	Atopic sensitization, cut of 3.5 kU/L										
	No	1252	90.3	549	78.3	968	80.6	476	75.1	3245	82.7
	Yes	134	9.7	152	21.7	233	19.4	158	24.9	677	17.3
Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0	

In table 40 the prevalence of atopic asthma and non-atopic asthma, by centre and by group is described. The definition of asthma used is doctor's diagnosis of asthma and the cut-off point for atopic sensitisation is ≥ 0.35 kU/litre. For atopic asthma the prevalence is lower in the farm group (3.1%) than in the farm reference group (5.4%). The Steiner school group has also a lower prevalence (5.7%) than their reference group (8.0%). The same is true for non-atopic asthma,

farm children (3.4%) compared to farm references (6.8) and Steiner school children (2.2%) compared to Steiner references (4.1%). Atopic asthma is less common than non-atopic asthma among farm and farm reference children, whereas the opposite is true among Steiner school children and their references.

Table 40. Prevalence of atopic (>0.35kU/L in Phadiatop) and non-atopic asthma (doctor's diagnosis), subdivided by group.

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Atopic asthma										
	No	1319	95.2	655	93.4	1114	92.8	573	90.4	3661	93.3
	Yes	43	3.1	38	5.4	68	5.7	51	8.0	200	5.1
	Not reporting	24	1.7	8	1.1	19	1.6	10	1.6	61	1.6
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	Nonatopic asthma										
	No	1315	94.9	645	92.0	1156	96.3	598	94.3	3714	94.7
	Yes	47	3.4	48	6.8	26	2.2	26	4.1	147	3.7
	Not reporting	24	1.7	8	1.1	19	1.6	10	1.6	61	1.6
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0

For all children with a positive Phadiatop test the following allergens were tested separately; grass, tree, horse, cat, *Dermatophagoides pteronyssinus* and *Lepidoglyphus destructor*. The results from these specific allergens are presented in Tables 41 – 46. The results from the screening for dietary allergens (fx5) are presented in table 47. For the test against grassmix, treemix, cat and horse allergens, as well as *D. pteronyssinus* and the food mix the farm children consistently show lower prevalences than all the other groups. Only for *L. destructor* the prevalence appeared slightly higher in the farm children. In the Steiner group the prevalence of test positivity appeared lower for most allergens than in their reference group.

Table 41. Number of positive tests against grassmix, subdivided by group. (unit kU/L)

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Grassmix										
	Neg	1207	87.1	544	77.6	976	81.3	477	75.2	3204	81.7
	Pos	177	12.8	157	22.4	223	18.6	157	24.8	714	18.2
	Not enough serum	0	0	0	0	2	0.2	0	0	2	0.1
	Missing	2	0.1	0	0	0	0	0	0	2	0.1
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	RAST class										
	less than 0.35	1207	87.1	544	77.6	976	81.3	477	75.2	3204	81.7
	0.35 - <0.7	20	1.4	18	2.6	48	4.0	20	3.2	106	2.7
	0.7 - <3.5	60	4.3	49	7.0	84	7.0	49	7.7	242	6.2
	3.5 - <17.5	17	1.2	26	3.7	49	4.1	32	5.0	124	3.2
	17.5 - <50	13	0.9	22	3.1	20	1.7	20	3.2	75	1.9
	50 - <100	8	0.6	25	3.6	13	1.1	14	2.2	60	1.5
	100 or more	5	0.4	12	1.7	8	0.7	17	2.7	42	1.1
	Not enough serum	0	0	0	0	2	0.2	0	0	2	0.1
Missing	56	4.0	5	0.7	1	0.1	5	0.8	67	1.7	
Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0	

Table 42. Numbers of positive tests against treemix, subdivided by group. (unit kU/L)

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Treemix										
	Neg	1254	90.5	600	85.6	1063	88.5	529	83.4	3446	87.9
	Pos	130	9.4	101	14.4	136	11.3	105	16.6	472	12.0
	Not enough serum	0	0	0	0	2	0.2	0	0	2	0.1
	Missing	2	0.1	0	0	0	0	0	0	2	0.1
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	RAST class										
	less than 0.35	1254	90.5	600	85.6	1063	88.5	529	83.4	3446	87.9
	0.35 - <0.7	20	1.4	19	2.7	36	3.0	20	3.2	95	2.4
	0.7 - <3.5	49	3.5	44	6.3	57	4.7	37	5.8	187	4.8
	3.5 - <17.5	21	1.5	21	3.0	21	1.7	25	3.9	88	2.2
	17.5 - <50	2	0.1	5	0.7	14	1.2	11	1.7	32	0.8
	50 - <100	0	0	5	0.7	4	0.3	5	0.8	14	0.4
	100 or more	0	0	2	0.3	2	0.2	3	0.5	7	0.2
	Not enough serum	0	0	0	0	2	0.2	0	0	2	0.1
Missing	40	2.9	5	0.7	2	0.2	4	0.6	51	1.3	
Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0	

Table 43. Numbers of positive tests against horse allergens, subdivided by group. (unit kU/L)

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Horse										
	Neg	1355	97.8	670	95.6	1150	95.8	608	95.9	3783	96.5
	Pos	28	2.0	31	4.4	50	4.2	26	4.1	135	3.4
	Not enough serum	0	0	0	0	1	0.1	0	0	1	0.0
	Missing	3	0.2	0	0	0	0	0	0	3	0.1
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	RAST class										
	less than 0.35	1355	97.8	670	95.6	1150	95.8	608	95.9	3783	96.5
	0.35 - <0.7	11	0.8	14	2.0	16	1.3	11	1.7	52	1.3
	0.7 - <3.5	10	0.7	8	1.1	19	1.6	12	1.9	49	1.2
	3.5 - <17.5	5	0.4	6	0.9	9	0.7	3	0.5	23	0.6
	17.5 - <50	1	0.1	2	0.3	3	0.2	0	0	6	0.2
	50 - <100	1	0.1	1	0.1	2	0.2	0	0	4	0.1
	100 or more	0	0	0	0	1	0.1	0	0	1	0.0
	Not enough serum	0	0	0	0	1	0.1	0	0	1	0.0
Missing	3	0.2	0	0	0	0	0	0	3	0.1	
Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0	

Table 44. Numbers of positive tests against cat allergens, subdivided by group. (unit kU/L)

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Cat										
	Neg	1331	96.0	653	93.2	1100	91.6	558	88.0	3642	92.9
	Pos	54	3.9	48	6.8	100	8.3	76	12.0	278	7.1
	Not enough serum	0	0	0	0	1	0.1	0	0	1	0.0
	Missing	1	0.1	0	0	0	0	0	0	1	0.0
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	RAST class										
	less than 0.35	1331	96.0	653	93.2	1100	91.6	558	88.0	3642	92.9
	0.35 - <0.7	18	1.3	11	1.6	33	2.7	13	2.1	75	1.9
	0.7 - <3.5	20	1.4	25	3.6	39	3.2	43	6.8	127	3.2
	3.5 - <17.5	14	1.0	4	0.6	12	1.0	13	2.1	43	1.1
	17.5 - <50	1	0.1	8	1.1	13	1.1	6	0.9	28	0.7
	50 - <100	1	0.1	0	0	2	0.2	1	0.2	4	0.1
	100 or more	0	0	0	0	1	0.1	0	0	1	0.0
	Not enough serum	0	0	0	0	1	0.1	0	0	1	0.0
Missing	1	0.1	0	0	0	0	0	0	1	0.0	
Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0	

Table 45. Numbers of positive tests against *Dermatophagoides pteronyssinus*, subdivided by group. (unit kU/L)

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	D.ptero										
	Neg	1246	89.9	579	82.6	986	82.1	504	79.5	3315	84.5
	Pos	139	10.0	122	17.4	214	17.8	130	20.5	605	15.4
	Not enough serum	0	0	0	0	1	0.1	0	0	1	0.0
	Missing	1	0.1	0	0	0	0	0	0	1	0.0
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	RAST class										
	less than 0.35	1246	89.9	579	82.6	986	82.1	504	79.5	3315	84.5
	0.35 - <0.7	23	1.7	13	1.9	26	2.2	12	1.9	74	1.9
	0.7 - <3.5	37	2.7	24	3.4	45	3.7	37	5.8	143	3.6
	3.5 - <17.5	31	2.2	30	4.3	47	3.9	24	3.8	132	3.4
	17.5 - <50	21	1.5	32	4.6	44	3.7	29	4.6	126	3.2
	50 - <100	19	1.4	18	2.6	36	3.0	22	3.5	95	2.4
	100 or more	8	0.6	5	0.7	16	1.3	6	0.9	35	0.9
	Not enough serum	0	0	0	0	1	0.1	0	0	1	0.0
Missing	1	0.1	0	0	0	0	0	0	1	0.0	
Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0	

Table 46. Numbers of positive tests against *Lepidoglyphus destructor*, subdivided by group. (unit kU/L)

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	L.destr										
	Neg	1308	94.4	671	95.7	1141	95.0	611	96.4	3731	95.1
	Pos	75	5.4	30	4.3	59	4.9	23	3.6	187	4.8
	Not enough serum	0	0	0	0	1	0.1	0	0	1	0.0
	Missing	3	0.2	0	0	0	0	0	0	3	0.1
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	RAST class										
	less than 0.35	1308	94.4	671	95.7	1141	95.0	611	96.4	3731	95.1
	0.35 - <0.7	19	1.4	13	1.9	24	2.0	11	1.7	67	1.7
	0.7 - <3.5	24	1.7	13	1.9	26	2.2	11	1.7	74	1.9
	3.5 - <17.5	28	2.0	2	0.3	6	0.5	1	0.2	37	0.9
	17.5 - <50	3	0.2	0	0	3	0.2	0	0	6	0.2
	50 - <100	0	0	1	0.1	0	0	0	0	1	0.0
	100 or more	1	0.1	1	0.1	0	0	0	0	2	0.1
	Not enough serum	0	0	0	0	1	0.1	0	0	1	0.0
Missing	3	0.2	0	0	0	0	0	0	3	0.1	
Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0	

Table 47. Numbers of positive tests against foodmix (fx5), subdivided by group. (unit kU/L)

		Farmer		Farmer ref		Steiner		Steiner ref		All groups	
		n	%	n	%	n	%	n	%	n	%
All centres	Foodmix										
	Neg	1246	89.9	599	85.4	1072	89.3	554	87.4	3471	88.5
	Pos	140	10.1	102	14.6	129	10.7	80	12.6	451	11.5
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0
	RAST class										
	less than 0.35	1246	89.9	599	85.4	1072	89.3	554	87.4	3471	88.5
	0.35 - <0.7	70	5.1	50	7.1	76	6.3	46	7.3	242	6.2
	0.7 - <3.5	62	4.5	40	5.7	44	3.7	31	4.9	177	4.5
	3.5 - <17.5	7	0.5	9	1.3	9	0.7	2	0.3	27	0.7
	17.5 - <50	0	0	1	0.1	0	0	1	0.2	2	0.1
	100 or more	1	0.1	2	0.3	0	0	0	0	3	0.1
	Total	1386	100.0	701	100.0	1201	100.0	634	100.0	3922	100.0

Dust sample analyses

In the study two different ways of collecting dust were used, the dust sock sampling by parents and the ALK dust sampling done by field workers. Both methods are described more thoroughly in the methods section. For 146 children, both ALK samples have been collected by fieldworkers, and sock samples by parents. In these children we compared the two methods of dust collection.

As can be seen in table 48 more mattress dust was collected by fieldworkers with ALK equipment as compared to parents using socks, whereas no such difference was found for living room samples, as can be seen in table 48. The levels of biological components in dust, collected with nylon socks by parents, appeared to correlate moderately to well ($r=0.4-0.9$) with levels in dust collected with ALK equipment by fieldworkers.

Table 48. Comparison of Sock sampling and ALK sampling.

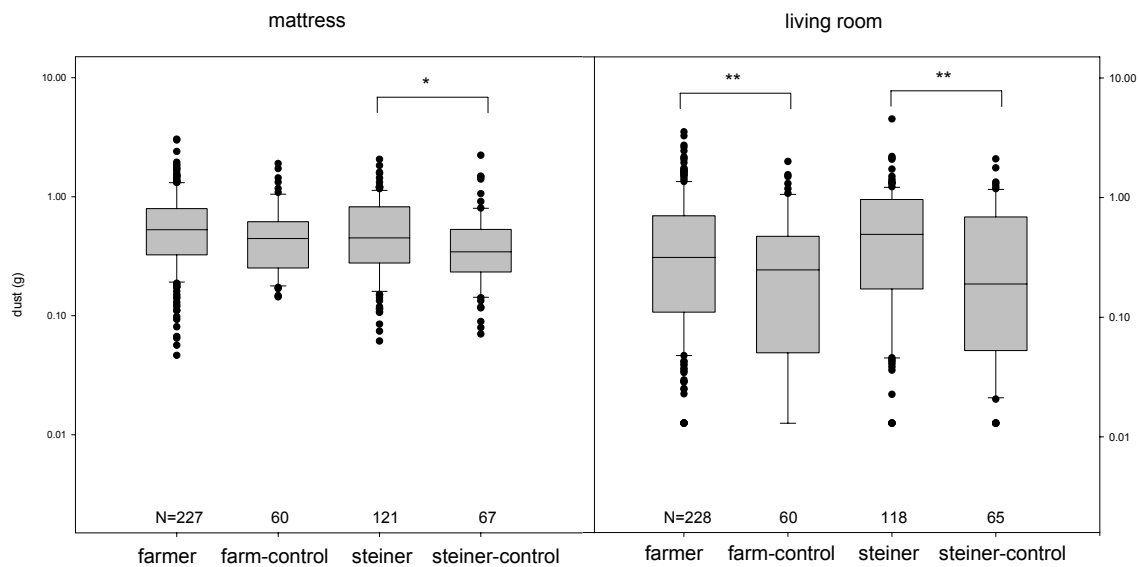
	N det	GM (g) (GSD), Sock	GM (g) (GSD), ALK	Correlation
Mattress	125	0.33 (3.22)	0.47 (1.95)	0.32
Living room	123	0.30 (3.20)	0.31 (3.40)	0.52

It has been suggested that the exposure to endotoxin is an important protective factor of farm environments. Little is known about exposure to other microbial components, exposure in anthroposophic families and differences between countries. The aim of the ALK analyses were to assess the levels and determinants of bacterial endotoxin, mould $\beta(1,3)$ -glucans and extracellular polysaccharides from *Aspergillus* and *Penicillium* (EPS) in the different groups and countries included in the study. As explained in the method section mattress and living room dust was collected in the homes of 271 farmers' children, 128 anthroposophic children and 64 and 67 of their respective references, as well as dust from the stables of farmers' children.

More living room and mattress dust was collected in homes of Steiner children as compared to their references (GM 0.34 versus 0.17 and 0.45 versus 0.35 g respectively, fig 1). For farm children, only amounts of living room dust were significantly higher as compared to farmer-references (GM 0.27 versus 0.18 g, fig 1). Figure 2 shows the distributions of microbial components per gram of dust for both sampling sites for each of the 4 groups. Farm children have higher levels of endotoxin (2.6-3.2 fold; $p<0.01$), EPS (2.4-3.1 fold; $p<0.01$) and glucans (1.2-1.3 fold; mattress $p<0.05$, living room n.s.) as compared to farmer references. Steiner children had somewhat higher levels than their references too (endotoxin 1.2-1.3 fold, n.s.; EPS 1.4-1.6 fold, $p < 0.05$; glucans 1.1-1.2-fold, mattress $p < 0.05$, living room n.s.) but differences were smaller and not always significant. For farmers' children and their references, the differences were 1.5-4.2 fold when levels were expressed per square meter, for Steiner children and their references, the differences were 1.6-2.5 fold when expressed per square meter (data not shown). The trends were consistent across countries, although levels varied considerably (fig. 3). For example, a 2.6 fold difference for endotoxin, 1.3 for EPS, and 2.2 for glucans was found between the countries with the highest and lowest levels per gram of mattress dust of farmers' children. For living rooms, those differences were 2.1, 1.7 and 1.6 fold respectively (not shown). Highest levels of EPS were observed in Switzerland (mattress) and Austria (living room), whilst highest levels of endotoxin and glucans were observed in Germany and Sweden respectively. The same was observed for levels per square meter, except for glucans, of which highest levels per square meter were not found in Sweden, but in Switzerland (mattress) and Germany (living room).

When expressed per square meter, differences between the countries with the highest and lowest mattress and living room levels of farmers' children were 2.0 to 4.7 fold (not shown).

Fig. 1. Boxplots of the dust amounts of mattress and living room samples. *, $p < 0.05$; **, $p < 0.01$.



High levels of endotoxin, EPS and glucans were found in stable dust (GM (GSD) 22.0×10^4 (3.0) EU/g; 9.0×10^5 (3.3) EPS units/g; 7.2×10^3 (2.0) $\mu\text{g/g}$ respectively). Those levels were respectively 5, 7 and 3-fold higher than levels of endotoxin, EPS and glucans in dust of living room floors of farmers' children. EPS and glucans in dust of mattresses and floors with carpets or rugs were correlated with the levels in stable dust (Pearson correlation coefficients for EPS per gram of dust 0.34/0.25 and for glucans 0.27/0.19 respectively), whilst no correlations were found for endotoxin.

The associations between farm characteristics and the observed levels in mattress dust of farmers' children after adjustment for home and family characteristics have also been calculated. Full time farming (EPS), goat keeping (EPS) or having chicken (glucans) increased the levels of at least one component in mattress dust. A direct connection between house and stable appeared to increase endotoxin levels in mattress dust, but this increase was not significant. For living rooms, full time farming (EPS, endotoxin) and goat keeping (EPS) were associated with higher levels of some of the components, whereas having a horse was associated with a lower level of EPS per gram of dust. The stable visit frequency of the child and the number of cows did not have any effect on levels in mattress or living room dust and these variables were left out of the final models.

Fig.2. Boxplots of the levels of microbial components per gram of dust in mattresses and living room floors. *, $p < 0.05$; **, $p < 0.01$.

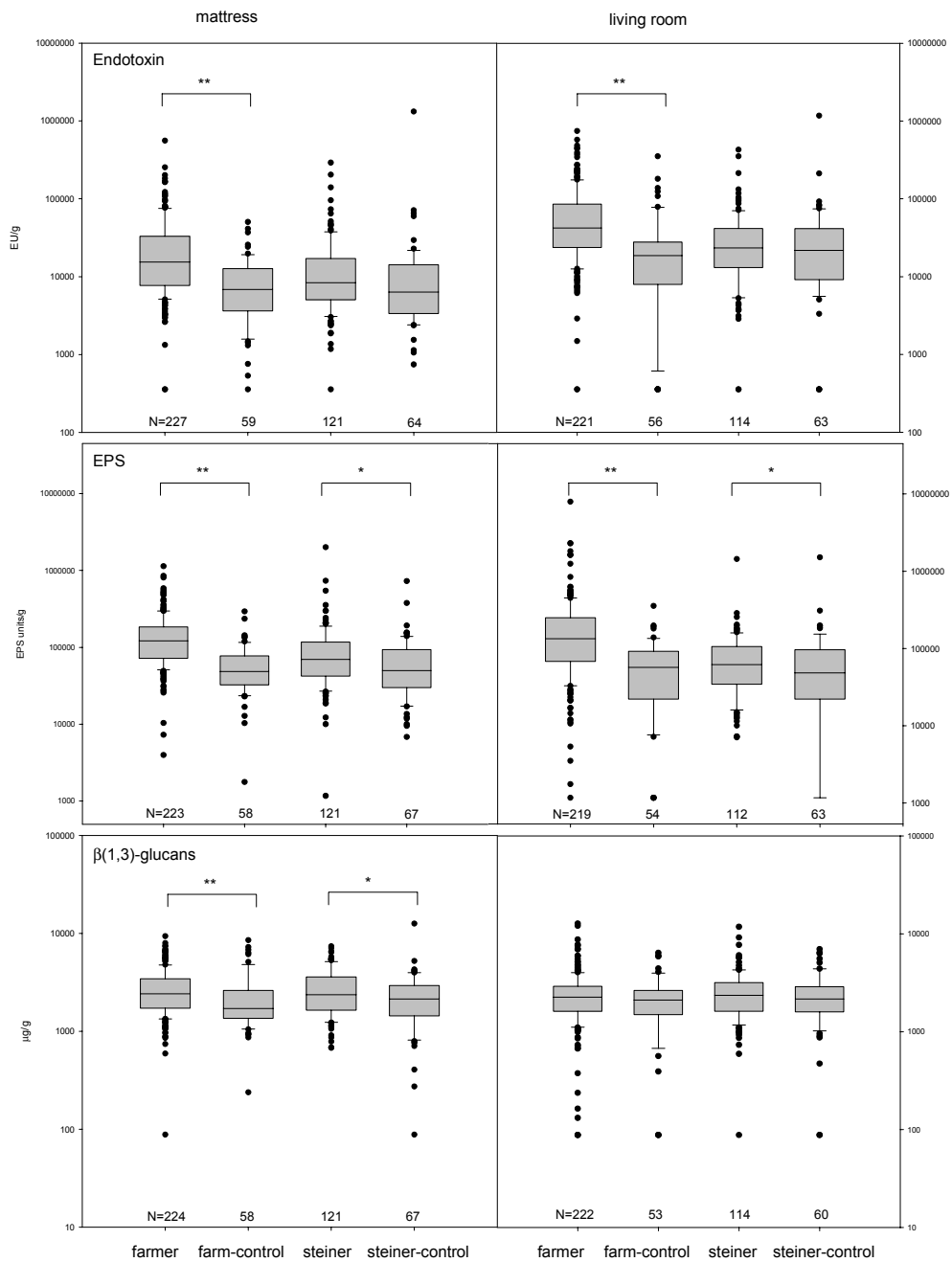
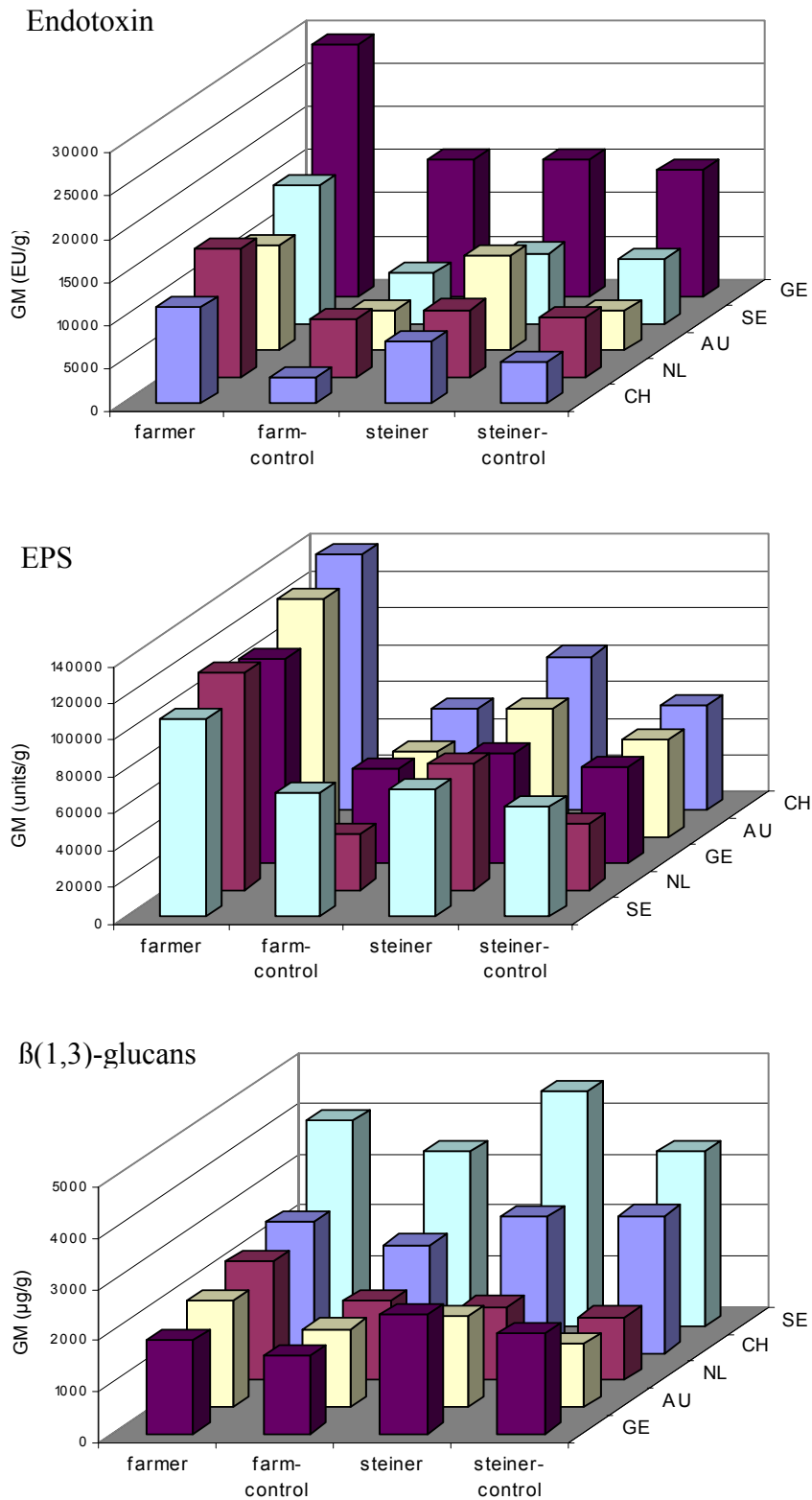


Fig. 3. Levels of microbial components in mattress dust by group and by country.



The associations between levels of microbial agents in house dust and atopy and wheezing in children have also been studied. Dust socks from 100 wheezers (atopic/non-atopic) and 100 references per country have been used in the analyses. Crude analyses without any adjustments. The atopic wheezers (n=270) had lower levels of endotoxins ($p=0.007$), lower levels of EPS ($p=0.006$), and lower levels of glucans than the reference group without wheezing (n=470). No such differences were found between the non-atopic wheezers and the references.

The here reported house dust analyses demonstrate that farmers' children and - to a lesser extent - Steiner children, are exposed to higher levels of endotoxin, EPS and glucans, than their respective references.

This is the first study showing elevated indoor exposure to fungal components in farming families and a correlation between levels in stable dust and in house dust.

Most significant determinants of levels per gram of dust (adjusted geometric means ratio ≥ 1.5) included group, country, contact to farm animals (endotoxin, EPS), dampness or moulds (EPS), using gas or wood for heating (endotoxin), type of floor covering (EPS), having both a cat and a dog (endotoxin), full time versus part time farming (endotoxin, EPS) and goat keeping (EPS). A smaller (adjusted geometric means ratio 1.3) but consistent association was found between EPS and age of the home.

Absolute levels differed substantially between countries, even after adjustment for differences in home characteristics. This cannot be due to differences in sampling and laboratory procedures, because we used standardized procedures and one laboratory for all analyses. The only difference in sampling procedures was the timing of fieldwork. Sweden, Switzerland and Austria sampled from August to October 2001, the Netherlands from January to June 2002 and Germany from the end of March to August 2001. But other studies in German homes found no effects of season (Bischof et al. 2002), temperature and relative humidity (Bischof et al. 2002) (Douwes et al. 1999) on endotoxin levels, so this is an unlikely explanation for the observed exposure differences. For EPS and glucans, no seasonal effects have been reported previously either (Chew et al. 2001). We have no explanation for the international exposure differences observed in this study.

This part of the study assessed differences in exposure to microbial components in house dust between farmers' and Steiner children and their respective references. The association between microbial exposure and allergic and airway diseases in these children will be further assessed in later analyses and presented elsewhere.

The data analysis regarding allergen levels in dust samples are ongoing and results will be submitted for publication in a scientific journal.

Faecal analyses

The normal gastrointestinal microflora helps to prevent potential pathogens from colonizing the mucosa. The intestinal flora is also supposed to have an impact on the development of the immune system, and is the major factor driving the maturation of the immune system in newborns (Bjorksten 1999). Studies on germfree animals indicate that establishment of a gut flora is a prerequisite for development of oral tolerance and immune deviation (Sudo et al. 1997). It has been postulated that lack of certain bacteria, colonisation with inappropriate strains or a limited bacterial turn over could delay the development of tolerance in infancy (Wold 1998).

Dietary habits and environmental conditions influence the intestinal pattern of the gut flora. An anthroposophic lifestyle is associated with a high intake of lactobacilli due to a diet that often contains fermented foods. Lactobacilli are harmless and even beneficial members of the normal intestinal flora, and are often used as “probiotics”, a live microbial feed supplement that beneficially affects the host by improving the intestinal microbial balance (Kalliomaki et al. 2001)). Furthermore, the use of antibiotics cause changes in the intestinal ecology (Brismar et al. 1993). As shown earlier in the report (table 20) the anthroposophic children have a restricted use of antibiotics. Contact with farm animals could possibly also cause changes in the intestinal flora.

In the first faecal analyses we compared the composition of the faecal bacterial microflora in samples from 23 Steiner school children with 23 Steiner reference children. DNA was extracted from the samples and 16S rDNA genes were amplified by PCR using primers directed towards bacteria or towards lactobacilli. The samples were analysed using terminal restriction fragment length polymorphism (T-RFLP). This methodology was used to determine the number and the relative abundance of terminal restriction fragments (TRFs), corresponding to individual populations or “taxonomic units”, in the samples. The resulting data was statistically analysed and correlated to diet, use of antibiotics and other information collected from the children. The results of this indicate a difference between the Steiner school group and the reference group with regard to their intestinal bacterial flora.

Analyses are also being done to assess the occurrence of *Brachyspira aalborgi* and *Lawsonia intracellularis* in the faecal samples. Preliminary analyses show, for the first time ever in humans, that *Brachyspira aalborgi* are found in children living on farms with pigs. On the other hand, *L. intracellularis* was not detected. Further extensive analyses of the faecal samples in the whole material are ongoing.

Bronchial hyperresponsiveness (BHR) analyses

One potentially weak point in several studies is that the estimated prevalence of asthma is usually based on questionnaire data. Thus, language, question phrasing, cultural differences as well as the translation of the questionnaire may decisively influence the responses. A translated written questionnaire may not be as precise as the originally validated questionnaire. For example, some languages do not have a term equivalent to 'wheeze'. Therefore it is desirable to have a more objective parameter for asthma related symptoms.

Bronchial hyperresponsiveness (BHR) has been shown to be closely associated with asthma and is less influenced by recognition or perception of symptoms and by linguistic or cultural differences. Although direct airway challenges using histamine and methacholine have been predominantly used for assessing BHR in field studies so far, indirect airway challenges appear to better differentiate asthma from other chronic lung diseases. Therefore, large population studies like the International Study of Asthma and Allergies in Children (ISAAC) have validated their questionnaire against bronchial response to hypertonic saline and exercise. For many participants in field studies, particularly for children, indirect challenges, involving more natural stimuli, are more appealing

The aim of the bronchial hyperresponsiveness part of PARSIFAL was to validate questionnaire information on asthma and wheeze against the bronchial response to hypertonic saline in a linguistically and culturally heterogeneous population. We particularly were interested in the association between BHR and health outcomes based on questionnaire responses in four groups with different cultural backgrounds.

We started our statistical analysis with the calculation of prevalence of positive BHR in the sample subgroups as well as their respective confidence intervals. This was followed by the epidemiological measures sensitivity, specificity, $LR+ [= \text{sensitivity}/(1 - \text{specificity}) = \text{ratio of (correct+)}/(\text{false+})]$ and $1/LR- [= \text{specificity}/(1 - \text{sensitivity}) = \text{ratio of (correct-)}]/(\text{false-})]$ for the questionnaire outcomes using BHR as the gold standard. We then calculated various coefficients to measure the association between the questionnaire data and the BHR outcome: the Spearman correlation coefficient rho, the kappa coefficient, the simple matching coefficient (proportion of cases with the same result – positive or negative – both for the questionnaire outcome and for BHR) and the Jaccard coefficient (proportion of agreement when cases with both results negative are omitted; i.e. the number of cases with both results positive relative to the number of cases with at least one of the two results positive). These coefficients measure slightly different aspects of the association between the questionnaire outcomes and the BHR outcome. As far as our aim of validating the information from the questionnaire was concerned, the main interest were not in their absolute value but in their respective values for 'farm children', 'farm reference children', 'Steiner school children' and 'Steiner reference children'. In other words, do these coefficients have more or less the same value in all four groups? The data analysis also included logistic regression analysis and chi-squared tests.

Although the BHR sample was planned to include 200 children from each centre, 25 'wheezers' and 25 'non-wheezers' in each of the four subgroups, as already mentioned, this was not always possible. Furthermore, missing values in the questionnaire data (particularly for 'doctor's diagnosis of asthma') reduced the sample further. Thus the different centres were not represented with the same frequency overall and in the four subgroups. To account for a possible 'Centre effect' when comparing these subgroups with respect to the epidemiological parameters, the measures of association and the odds ratios, weighted analysis was used. For example, for 'doctor's diagnosis of asthma', the Swedish contingent constitutes 18.9% of the overall sample

with non-missing values, but 21.6% of the ‘farm children’ and only 13.2% of the ‘farm reference children’. Thus the Swedish data for ‘farm children’ was weighted down (weighting factor $18.92/21.56 = .8776$) and for ‘farm reference children’ was weighted up (weighting factor $18.92/13.21 = 1.4324$). Due to different numbers of missing, there were different weights for the analysis of the ‘wheeze’ data and the ‘doctor’s diagnosis of asthma’ data.

778 children completed the HS challenge, 9 with an initial FEV₁ %predicted < 75% which after Salbutamol increased by at least 15% and 769 with an initial FEV₁ %predicted ≥75%.

Prevalence of ‘wheeze’, ‘doctor’s diagnosis of asthma’ and BHR by centre and by subgroup are given in table 49.

Table 49: Prevalence of “Wheeze”, “Asthma diagnosis”^{*} and bronchial hyperresponsiveness (BHR) to the hypertonic saline challenge in 778 children by centre and by subgroup

	Questionnaire				BHR			95% CI	
	“Wheeze”		“Asthma diagnosis”		valid	n	%		
	valid N	% pos.	valid N	% pos.	N	pos.	pos.	lo	hi
All (N=778)	774	41.0	703	27.0	778	228	29.3	26.2	32.6
Centre:									
Austria (N=143)	141	34.0	125	19.2	143	20	14.0	9.2	20.6
Germany (N=207)	207	44.0	185	30.3	207	71	34.3	28.2	41.0
Holland (N=114)	113	47.8	103	35.9	114	36	31.6	23.8	40.6
Sweden (N=144)	143	38.5	133	24.1	144	25	17.4	12.1	24.5
Switzerland (N=170)	170	40.6	157	26.1	170	76	44.7	37.4	52.2
Group:									
Farmers’ children (N=183)	181	38.1	167	22.2	183	46	25.1	19.4	31.9
Steiner children (N=243)	241	45.2	215	28.8	243	72	29.6	24.2	35.7
Farmer reference children (N=173)	173	38.2	159	24.5	173	54	31.2	24.8	38.5
Steiner reference children (N=179)	179	40.8	162	32.1	179	56	31.3	24.9	38.4

*“Wheeze” is defined as wheeze in the last 12 months and “Asthma diagnosis” is defined as a doctor’s diagnosis of asthma at least once or a doctor’s diagnosis of spastic, obstructive or asthmatic bronchitis more than once.

When comparing ‘farm children’, ‘Steiner school children’, ‘farm reference children’ and ‘Steiner reference children’ on the basis of the epidemiological parameters we saw that the positive likelihood ratios for ‘wheeze’ were between 1.94 and 2.37 and the negative likelihood ratios were between 1.73 and 2.14. For ‘doctor’s diagnosis of asthma’ the respective values were between 1.96 and 3.18 (LR+) and between 1.28 and 1.70 (1/LR-). The respective confidence intervals for the four subgroups were heavily overlapping (Table 50). However there were indications of slight differences between ‘farm children’ and ‘farm reference children’. For ‘wheeze’ and BHR Spearman’s rho was 0.26 for ‘farm children’ and 0.33 for the ‘farm reference children’ and for ‘doctor’s diagnosis of asthma’ 0.33 and 0.19 respectively (Table 51). A similar difference for these two subgroups was seen in the odds ratios (3.60 vs. 5.08 for ‘wheeze’, 5.41 vs. 2.76 for ‘doctor’s diagnosis of asthma’) (Table 52). However these differences were not statistically significant ($p=0.454$, $p=0.203$ resp.) Also the p -values for the interaction of BHR and ‘wheeze’ / resp. BHR and ‘doctor’s diagnosis of asthma’ (resp.) were 0.818 and 0.620 (Table 52).

Table 50: Sensitivity, specificity and likelihood ratios* of “Wheeze” (N=774) and “Asthma diagnosis” (N=703) with respect to bronchial hyperresponsiveness to the hypertonic saline challenge

	Sensitivity (%)			Specificity (%)			LR+			1/LR(-)			
	95% CI			95% CI			95% CI			95% CI			
	lo	hi		lo	hi		lo	hi	lo	hi		lo	hi
“Wheeze”													
Farmers’ children	59.9	45.6	72.6	69.2	61.0	76.4	1.94	1.38	2.75	1.73	1.19	2.49	
Steiner children	69.1	57.7	78.5	66.0	58.6	72.8	2.03	1.57	2.64	2.14	1.49	3.06	
Farmer reference children	60.2	46.5	72.4	74.6	66.2	81.5	2.37	1.62	3.45	1.87	1.32	2.66	
Steiner reference children	65.9	52.8	77.0	71.4	62.9	78.6	2.30	1.64	3.22	2.09	1.43	3.07	
All	64.4	57.9	70.3	69.9	66.0	73.6	2.14	1.82	2.51	1.96	1.64	2.36	
“Asthma diagnosis”													
Farmers’ children	46.0	31.9	60.8	85.5	78.3	90.6	3.18	1.86	5.44	1.58	1.19	2.11	
Steiner children	51.7	40.1	63.1	81.1	74.0	86.6	2.27	1.82	4.10	1.68	1.30	2.17	
Farmer reference children	36.5	24.3	50.7	81.4	73.2	87.5	1.96	1.14	3.37	1.28	1.02	1.62	
Steiner reference children	53.6	40.0	66.7	78.8	70.3	85.3	2.53	1.62	3.92	1.70	1.24	2.32	
All	47.5	40.8	54.3	81.8	78.1	84.9	2.60	2.06	3.29	1.56	1.36	1.78	

*LR+=sens./(1-spec.)=ratio of (correct+)/(false+); 1/LR- =spec/(1-sens)=ratio of (correct -)/(false -)

Table 51: Measures of association/ similarity* between “Wheeze” (N=774)/ resp. “Asthma diagnosis” (N=703) and BHR

	“Wheeze” & BHR				“Asthma diagnosis” & BHR			
	Spearman’s rho	Simple Matching	Jaccard	Kappa κ	Spearman’s rho	Simple Matching	Jaccard	Kappa κ
Farm children	0.26	0.67	0.32	0.25	0.33	0.76	0.32	0.33
Steiner school children	0.32	0.67	0.39	0.31	0.34	0.72	0.37	0.36
Farm reference children	0.33	0.70	0.38	0.33	0.19	0.68	0.25	0.19
Steiner reference children	0.36	0.70	0.40	0.35	0.32	0.71	0.36	0.32
All	0.32	0.68	0.37	0.31	0.30	0.72	0.33	0.30

*For coefficient definitions see Section:Statistics

Table 52: Odds ratios (BHR pos/neg) for “Wheeze” (N=774) and “Asthma diagnosis” (N=703)

	“Wheeze”			“Asthma diagnosis”		
	OR	95%CI		OR	95%CI	
		lo	hi		lo	hi
All	4.20	3.03	5.53	4.05	2.84	5.78
Farm children	3.60	1.90	6.81	5.41	2.55	11.49
Steiner school children	3.72	2.14	6.46	4.47	2.37	8.43
Farm reference children	5.08	2.61	9.87	2.76	1.35	5.66
Steiner reference children	4.82	2.52	9.20	3.96	1.91	8.19
p-value interaction BHRxsubgroup	n.s. (0.818)			n.s. (0.620)		
p-value Farm vs. Farm reference	n.s. (0.454)			n.s. (0.203)		
p-value Steiner vs Steiner reference	n.s. (0.546)			n.s. (0.805)		

The findings presented show a reasonable correlation between the response to the hypertonic saline as an objective marker of asthma and the health outcome questions (‘wheeze’ and ‘doctor’s diagnosis of asthma’) based on the information from the questionnaire. This correlation does not differ substantially among the four subgroups studied in this survey. These similar associations suggest that differences in the prevalence of asthma between ‘farm children’, ‘Steiner school children’, ‘farm reference children’ and ‘Steiner reference children’ based on questionnaire information are reliable. We feel confident that different languages, interpretation of the concept of the health outcome questions or cultural features do not bias questionnaire responses with respect to ‘doctor’s diagnosis of asthma’ and ‘wheeze’.

However, for some comparisons there are indications of slight differences. In contrast to all the other subgroups, the ‘farm children’ show a slightly lower correlation for ‘wheeze’ and BHR. Since the association between ‘doctor’s diagnosis of asthma’ and BHR is much better we assume that the farming population has a clearer understanding of the term ‘asthma’ than of ‘wheeze’. On the other hand the ‘farm reference group’ show a slightly lower correlation for ‘doctor’s diagnosis of asthma’ and BHR than the other subgroups. We have no reasonable explanation for this finding. These differences are not statistically significant and they are unlikely to explain observed differences in the prevalence of ‘doctor’s diagnosis of asthma’ and ‘wheeze’ among the various populations.

Although we think that BHR is an objective marker of asthma, which is relatively easy to assess, we experienced some problems in performing the hypertonic saline challenge in the field. This particularly applied to the nebulisation rates. In several cases we found low nebulisation rates. In previous studies it had been shown that the nebulisation rate can strongly influence the result of an airway challenge. We tried to standardize the procedure of the hypertonic saline challenge as much as possible by using a detailed protocol and organizing fieldworker trainings on the procedure. However, particularly in one centre for a substantial number of subjects the nebulisation rate was lower than 1.5 ml/min, which is considered to be the required minimum. To control for this potential limitation in the technique we analysed our data with respect to the

nebulisation rate. If we only take subjects with a nebulisation rate of at least 1.5 ml/min, the specificity for 'doctor's diagnosis of asthma' for the 'farm children' is significantly higher than for the 'farm reference children' (93.3% vs. 83.5%; $p = 0.047$). This increased specificity could contribute to a lower prevalence of asthma in the farming population, but it is unlikely that it fully explains the lower risks.

Another factor affecting the association between BHR and 'wheeze' is the time lag between questionnaire response and the hypertonic saline challenge. 25% of the HS challenges were performed within 3.5 months but for 20% there was a time lag of more than 10 months. For the former group the association between BHR and 'wheeze' was much stronger than for latter (Spearman's rho: 0.470 vs. 0.321) However, the time lag distribution did not differ substantially among the four groups.

So far different methods for validating questionnaires on asthma have been used: comparing the questionnaire response to a bronchial challenge test, comparing the questionnaire response with a previous doctor's diagnosis of asthma and comparing questionnaire response with responses from another questionnaire. Validation of survey tools is usually done by comparing the results from the tool to the 'gold standard' test. Various studies reflect the lack of a true gold standard for diagnosing asthma. In clinical studies, the presence and degree of BHR correlate well with the prevalence of a physician's diagnosis of asthma and the severity of the disease. Airway inflammation is likely to play an important role in the pathogenesis of airway responsiveness and asthma. However, inflammatory cell recruitment to the epithelium which is a characteristic of late asthmatic reaction and thus of BHR is not unique to or specific for asthma. BHR has been shown to persist independently of asthma. Bronchial hyperresponsiveness is only one aspect of the complex and composite syndrome called 'asthma'.

One other survey validated the self-completed ISAAC questionnaire and the ISAAC video questionnaire against bronchial hyperreactivity in 13-14 year old Korean children. 499 children completed both questionnaires and one year later the children performed the hypertonic saline challenge. 3.8% of the children were hyperresponsive. With BHR as the 'gold standard' the specificity for 'asthma ever' was 63% (as compared to 82% in our study) and the sensitivity 61% (as compared to 48% in our study). The authors speculate that the low specificity in their study is due to a misunderstanding concerning 'asthma' in the Korean population and that the responses to the questionnaire were possibly influenced by culture and language.

Gibson et al. validated the ISAAC questionnaire against BHR in adolescents from a mixed ethnic background. 457 students aged 13.5 years (SD 1.3) completed the video questionnaire and approximately one week later a subgroup of these (169) completed the written questionnaire and performed a hypertonic saline challenge. The specificity of the question 'wheeze' in the last 12 months compared to BHR to hypertonic saline was 66% and the sensitivity 88%. This is very similar to our results in children, who performed the hypertonic saline challenge within 3.5 months of the questionnaire (specificity 67% and sensitivity 83%).

In conclusion we found no substantial differences in the association between BHR to hypertonic saline and questionnaire information on 'asthma' and 'wheeze' among 'farm children', 'Steiner school children', 'farm reference children' and 'Steiner reference children'. This suggests that the information of the questionnaire in culturally different populations, as validated in our study, is reliable.

Validity and reproducibility of the food questionnaire

The role of diet in disease etiology has become of increasing interest over the past decade. More recently, research has focused on the relevance of children's diet as a cause or contributor to childhood or chronic diseases.

Assessing diet in children is particularly challenging, as children are generally less aware of ingredients and components of consumed dishes and meals. Furthermore, children are – depending on their age – less able to complete a self-administered questionnaire (Livingstone et al. 2000). Conversely, if parents complete the questionnaire on their child's behalf, an additional element of uncertainty may be introduced if the child consumes foods or meals away from home and due to parent's unawareness of the children's snacking behaviours. When studying the child's food intake in a home setting, several researchers have reported that parents actually can be reliable reporters of their children's food intake (Klesges et al. 1987; Eck et al. 1989; Baranowski et al. 1991).

Dietary information collected in an epidemiologic study is affected by measurement error. The most commonly used dietary assessment instruments are food frequency questionnaires, diet diaries, and 24-h (24-hour) recalls (Rockett et al. 2003). In the PARSIFAL-study, dietary information was collected through parental reports. The diet part of the questionnaire focused on the consumption of foods with particular allergenic or protective potential. We present here a food-based assessment of validity of the food questionnaire with a 24-h recall and of the reproducibility with a second administration of the diet questionnaire.

The dietary component of the PARSIFAL questionnaire includes questions on the frequency of current average consumption of 11 home-grown and 17 store-purchased foods. The foods were selected based on their potential allergenic or protective potential. These foods include milk, butter, other dairy products (yogurt, cottage cheese), margarine, eggs, meats, vegetables, fruit, whole grain products, peanuts and fermented vegetables. Response options are restricted to four categories: never, less than once per week, one to six times per week, or once a day or more. Home-grown and store-purchased foods were assessed separately to capture differences in preservation levels.

The questionnaire also asks at which age the child first consumed each of 10 foods, whether foods are consumed from largely conventional, biologic (organic), or biodynamic production, on vegetarian, lacto-, or lacto-ovo vegetarian dietary restrictions. Biologic or organic production of foods avoids the use of chemical pesticides and fertilizers, the use of antibiotics in cattle, and prescribes 80% ecologic feed and a free-range living environment for cattle. Biodynamic production is more restrictive eliminating even natural pesticides and fertilizers (using compost instead for fertilization) and 100% ecologic animal feed.

The dietary items included in the questionnaire do not provide a complete representation of the child's diet, thus nutrients or total caloric intake cannot be calculated. The dietary component of the questionnaire was kept short to avoid burden on the participant.

The validity of the dietary information provided by the parents as proxies for their child was evaluated using the 24-h recall administered to a random sample of about 100 participants from each country. Of the participants from each country, about equal numbers were sampled from the farmers' children, the Steiner school children, and the farmers and Steiner School reference children, respectively. The goal of the 24-h recall was to evaluate whether the foods reported on the main questionnaire were actually consumed by the children. During the interview, the

interviewer asked the parent together with the child to recall all food items and beverages consumed during the previous 24 hours. The interviewer also inquired whether the prior 24 hours represented a typical day with respect of the diet of the child. The 24-h recall always pertained to diet on a weekday. After the interview was completed, the interviewer entered the information into a database that was restricted to the foods of interest from the main questionnaire. Thus the database established for the 24-h recall correlated directly with the database from the dietary questionnaire.

Reproducibility of the frequency of foods consumed was evaluated in an additional subgroup of participants. Families who consented to collect a faeces sample of the child were asked to answer a supplementary questionnaire. This supplementary questionnaire included the same dietary questions that were part of the main questionnaire pertaining to the child's diet during the previous three months. The main purpose of the supplementary questionnaire was to relate food intake to intestinal microflora. When studying that connection it is important to have information about the child's most recent consumption of different foods and that is why the dietary questions were distributed once again. The supplementary questionnaire was mailed to the participating families and returned when the fieldwork staff collected the sample.

The 24-h recall conducted by our interviewers also included a reproducibility component. The question when the child had consumed a selected list of foods for the first time from the main questionnaire was repeated during the 24-h recall. Furthermore, the questions pertaining to the consumption of largely conventional, biologic, or biodynamic products, vegetarian, lacto-, or lacto-ovo vegetarianism, and use of vitamin supplements were repeated in the same format as they appeared on the main questionnaire.

The validity of reported frequency of consumption of home-grown and store-purchased foods was assessed using the positive predictive value (PPV) and the negative predictive value (NPV). PPV and NPV were better defined in our dataset than sensitivity and specificity. The PPV was defined as the proportion of participants who reported consumption of food during the previous 24 hours among those who reported current consumption at least once per day on the main questionnaire. The NPV was defined as the proportion of participants who did not report consumption of food during the previous 24 hours among those who reported never consuming the food on the main questionnaire. Logistic regression was used to assess validity while adjusting for age, gender, country, and study group. The main questionnaire response was used as independent variable maintaining frequencies of consumption to predict the response from the 24-h recall. Agreement of the two methods was expressed as a chi-square statistic (Wald test).

To assess reproducibility, the concordance of responses was evaluated. We calculated the proportion of participants whose responses were the same for the repeated applications of the questionnaire. We also calculated the proportion of response which differed by one category or less. A weighted Kappa statistic was computed to assess the test-retest reliability between the questionnaires. Kappa statistics have been arranged hierarchically and evaluated in a 6 grade-scale; below 0=poor, 0.00-0.20 slight, 0.21-0.40=fair, 0.41-0.60=moderate, 0.61-0.80=Substantial, and 0.81-1.00=almost perfect, according to Landis (Landis et al. 1977).

A total of 493 children were included in the validation component of this study. The sample included 255 girls and 237 boys, and the mean age was 8.8 years. Of the sample, 124 were farmers' children, 122 children from Rudolf Steiner schools, 122 farmer reference children, and 125 Rudolf Steiner School reference children. There was no important age difference in participants between country and group, although the Rudolf Steiner School children were somewhat older (mean age 9.5 years) than the farmers' children (mean age 8.7 years).

The distribution of consumption of store-purchased and home-grown foods among the entire cohort is provided in table 53 and 54. The food intake differed considerably between the groups (data not shown). For example, farm milk (heated and not heated) was consumed daily by 52% of the farmer children compared to 5% of the Steiner school children and 2-4% of the reference children. Daily intake of butter, eggs, meat and sausages also differed substantially between the groups.

Table 53. Frequency of responses to the question on frequency of consumption of store-purchased foods on the main questionnaire among the entire study population (n=14.893)

Store purchased foods	Number of responses	Frequency of responses (%)				Missing
		Never	<1/w	1-6/w	1+/day	
Milk (3%)	13,207	39.2	13.6	21.9	25.3	11.3
Milk (0.5%-1.5%)	13,274	52.5	14.5	14.5	18.5	10.9
Long-life milk	12,817	68.7	11.0	10.0	10.3	13.9
Other milk	13,310	69.1	5.9	5.8	19.2	10.6
Butter	14,242	8.7	12.3	32.1	46.8	4.4
Margarine	13,933	37.8	20.1	21.4	20.7	6.5
Other dairy products	14,376	4.0	16.2	57.5	22.4	3.5
Olive oil	14,093	25.2	24.6	38.9	11.3	5.4
Other vegetable oil	13,752	14.8	28.3	48.2	8.7	7.7
Meat or sausage	14,436	5.2	16.4	60.3	18.2	3.1
Eggs	14,292	12.9	35.0	50.5	1.7	4.0
Potatoes	14,350	7.3	11.2	74.4	7.2	3.7
Vegetables	14,383	4.0	10.5	51.4	34.1	3.4
Fruit	14,379	2.1	8.5	45.1	44.4	3.5
Peanuts*	11,136	15.8	76.1	7.3	0.9	25.2
Whole grain products	14,334	6.9	24.1	43.0	26.1	3.8
Fermented vegetables	13,458	51.7	37.7	9.5	1.1	9.6

* Not assessed in the Netherlands

Table 54. Frequency of responses to the question on frequency of consumption of home-grown foods on the main questionnaire among the entire study population (n=14.901)

Homegrown foods	Number of responses	Frequency of consumption (%)				Missing
		Never	<1/w	1-6/w	1+/day	
Farm milk, heated	12,081	79.5	5.8	5.7	9.0	18.9
Farm milk, not heated	12,057	82.6	5.1	5.0	7.4	19.0
Butter	11,872	78.3	4.9	6.6	10.2	20.3
Other dairy products	11,862	74.3	7.0	12.9	5.8	20.4
Meat or sausage	11,934	66.2	8.1	18.0	7.6	19.9
Eggs	12,066	53.8	19.3	25.0	2.1	19.0
Potatoes	11,986	56.1	9.2	29.3	5.4	19.5
Vegetables	12,131	41.5	17.2	29.3	12.0	18.6
Fruit	12,058	41.5	17.7	26.6	14.2	19.0
Whole grain products	11,796	73.1	8.5	11.5	6.9	20.8
Fermented vegetables	11,499	83.6	11.6	4.1	0.7	22.8
Water from own well	11,940	79.5	2.7	2.6	15.2	19.8

The validity of reported frequencies of consumption of store-purchased foods was then tested. The PPV (PPV=Positive predictive value: Proportion of participants who reported consumption of food during the previous 24-hours among those who reported current consumption at least once per day on the main questionnaire) was highest for foods consumed with high frequency such as vegetables, fruits, meats and milk. The NPV (NPV=Negative predictive value: Proportion of participants who did not report consumption of food during the previous 24-hours among those who reported never consuming the food on the main questionnaire) was above 80% for most foods exception for fruits and vegetables. The Wald test indicated good validity for most foods with the exception of eggs, peanuts and fermented vegetables. Among homegrown foods, the PPV was somewhat lower than among store-purchased products. The PPV was highest for milk and fruit. The NPV of consumption of homegrown foods was above 90%. No important differences were found in the validity of responses between the five countries participating or between the four groups of children.

In total, the reproducibility part included 424 children whose parents answered both the main and the supplementary questionnaire. Of these, 222 were children of farmers, 105 Rudolf Steiner school children, 50 farmer reference children and 51 Rudolf Steiner school reference children. Their mean age was 8.8 ± 1.9 years and 223 (52%) were boys. There were no important differences in age between the groups. For 358 participants the mother answered both questionnaires, for 24 by the father, and for 16 by the child itself. The remaining questionnaires were completed by different responders. The time between answering the two questionnaires had a median of 11 months (median range: The Netherlands: 9 and Switzerland: 14 months).

Reproducibility of food consumption then calculated. Among store-purchased foods, milk, potatoes, vegetables and peanuts had the highest and butter and whole grain products the lowest proportion of concordant responses. The weighted Kappa statistic was substantial for milk and fair for peanuts. Among home-grown products, concordance was high for milk and fermented vegetables and below 60% for fruits and vegetables. The weighted Kappa statistic was substantial for milk and fair for fermented vegetables and whole grain products. Reproducibility of self-reports of water supply from own well was almost perfect.

Concordance between the questionnaire and the 24-h recall ranged between 35% for nuts and 64% for meat and sausage consumption and responses were within one response category for 62% of respondents on fermented vegetable consumption and for 96% of respondents for consumption of meat and sausages. Reproducibility of reported consumption of milk during the first year of life was concordant among 71% of participants for store purchased milk and for 85% for farm-produced milk. Vegetarian eating habits were highly reproducible. Reproducibility of reported consumption of home-grown foods, organic food, and water from a home well were above 65%. Kappa statistics for individual foods consumed for the first time are estimated to be fair to moderate. No important differences by age and gender were observed.

Overall the calculations show moderate to acceptable validity and moderate to substantial reproducibility of parents' reports of their 5-13 year old children's diet. The range of values observed in our study is within the range reported from other studies on validity and reproducibility of diet reports in children. Our study differed from most previous studies since validation was performed on a food rather than a nutrient basis and questions were limited to a selected number of foods, which were thought to be of particular relevance for the study endpoints.

A number of studies have been conducted to assess the validity of diet questionnaires completed by parents on behalf of their child. Only two prior studies paralleled our design of validating a food frequency questionnaire (FFQ) with 24-h recalls (Treiber et al. 1990; Stein et al. 1992); both validations were based on nutrients. A comparison of an FFQ capturing the diet of 55 U.S. children ages 3-5 years for the previous 3 months completed by their parents with a 24-h recall indicated high consistency: carbohydrate and protein intake as percentage of total energy differed by only 1% between the 2 methods (Treiber et al. 1990). Stein and colleagues observed correlations between estimated mean nutrient intake from two FFQ and four 24-h recalls administered to 224 parents of 4- to 5-year old children in New York City of 0.48 for total calories and 0.35 for total fat which is in the range reported for adult validation studies on diet (Stein et al. 1992).

Byers and colleagues compared the accuracy of parents' report of their children's consumption of fruits and vegetables with serum levels of carotenoids, vitamins C, A, and E (Byers et al. 1993). An FFQ was administered to 97 parents of children age 6- to 10-years old asking about the child's usual dietary intake over the previous 3 months. The dietary reports of consumption of 35 fruits and vegetables indicated Spearman rank order correlations of 0.30 with serum carotenoids and 0.34 with serum vitamin C.

In a study conducted in Texas including 66 mothers of 3- to 5-year old children, the accuracy of a 24-h recall of the child's diet by the mother was evaluated (Baranowski et al. 1991). A comparison with a detailed record of the previous day's consumption obtained by an observer resulted in a 64.8% agreement with the maternal recall. In a similar study including 33 children, an average correlation of $r=0.65$ was found between a 24-h dietary recall of the parent of a child aged 24 to 48 months and direct dietary intake recording by an observer (Klesges et al. 1987). A study conducted in Northern Ireland including 78 children aged 3-18 years indicated that dietary recall by mothers for their children was more representative of habitual dietary intake than 7-day weighed dietary intake records, which tend to underestimate energy intake as measured by doubly labelled water (mean diet histories were 98 to 114% of total energy expenditure) (Livingstone et al. 1992).

The goal of the validation study within PARSIFAL was to evaluate whether the dietary items reported on the main questionnaire were indeed consumed. Recall of diet during the previous 24 hours is affected by intra-individual and seasonal variation but population means are reliable (Willet 1998). Thus, assessing the validity of the diet reports with a 24-h recall should be informative. Intra-individual variation and measurement error affect all self-reports of diet. Hence, statistical measures of agreement between two different types of dietary assessment methods or the repeated application of the same method can be expected to be modest (Willet 1998).

Optimally, the 24-h recall should be administered as closely as possible in time to the main questionnaire to minimize true changes in diet affecting the results. Seasonal variation in food supply is like to have impacted our estimates. Fruit and vegetable consumption varies by season, in particular, the availability of home-grown produce. Our main questionnaires asked about current frequency of consumption. Thus, the time-lag between administration of the main questionnaire and the 24-h recall or a second diet questionnaire might have introduced additional within-person-variation which could have affected measures of validity and reproducibility for these foods. Indeed, we found validity and reproducibility generally to be higher among frequently consumed foods and among foods whose consumption does not vary by season. Positive predictive values of store-purchased foods were higher than of home-grown foods due to the higher prevalence of consumption.

Assessments of dietary intake among young school children possess a particular challenge if the children are too young to complete the questionnaires themselves. While parents probably know general dietary habits and preferences of their child, it has been found that particularly snack foods and sodas are underreported by the parents as they are not always aware of the child's behaviour (Potischman et al. 1998). Our study, however, focused on the consumption of a main food items and food groups.

Few reproducibility studies of children's food intake registered by FFQs completed by parents were found. Some of them calculate a mean from the FFQs and compare with other dietary assessment methods and do not report the test-retest reliability (Shea et al. 1991; Stein et al. 1992). Despite this, there are studies matching this reproducibility study and that also show similar results. One is conducted by Arnold et al (Arnold et al. 1995), who investigated the reproducibility of a food frequency questionnaire (FFQ) used on girls aged 7-12 years. They found the highest correlation for intake of fibre, vitamin C and B₂. In the present study, when looking at store-purchased foods, the highest total agreement was seen for potatoes and vegetables (fibre, vitamin C), peanuts (fibre, vitamin B₂) and milk and eggs (vitamin B₂) which is in line with the study by Arnold.

Treiber et al (Treiber et al. 1990) compared the test-retest reliability of a FFQ on food intake in preschool children. The FFQ assessed the food intake over 3 months and was completed by the parent with one week in between. They found very good concordance between intake recorded in the first and the second FFQ and the correlation varied between 0.42 – 0.83 for macro- and micro nutrients. The mean correlation obtained from the two FFQs was 0.67. This study demonstrates that parents possess good information about their child's food intake and that they can be reliable sources of information in this area.

We also examined a reproducibility component in the maternal recall of the child's age at introduction of a number of foods and found acceptable reproducibility. We could not test the validity of these reports because we had no information about the correct age of introduction. We identified one study that examined the validity of maternal recall of infant feeding after 8 years (Vobecky et al. 1988). The mothers of 95 children were asked to recall the age of their child at introduction of cereal and meat. The reports were compared to data recorded at baseline. The correlation coefficients were 0.35 for age at introduction of cereals and 0.16 for age at introduction of meat, questioning the validity of the recall.

We conclude that the validity of the main questionnaire should be sufficiently accurate for most of the foods when used in diet-disease analyses. Repeated administration of the diet questions indicated moderate to substantial reliability for most foods. Validity and reproducibility were generally higher among frequently consumed foods and among foods whose consumption does not vary by season.

Farm analyses

Of the farm children in PARSIFAL 95% of their parents run a farm, compared to the reference groups where almost none run a farm (tables 32-34). Further, not surprisingly, children to farmers were in contact with farm animals during both their first and second year of life to a much larger extent than the other groups and also the mothers to the farm children were in contact with farm animals during the pregnancy to a much larger extent than the other mothers. The farm children also had mothers who smoked less during pregnancy (table 17), had parents with slightly less education (table 12) and more older siblings than their reference group (table 13).

As shown in tables 35 – 40 the farm children have, compared to their reference group, as well as compared to Steiner school children and their references, a lower crude prevalence of Dr's diagnoses of asthma, current wheezing, coughing at night, atopic dermatitis, Dr's diagnoses of hay fever, current hay fever), as well as atopic sensitisation. In table 55 odds ratios are provided, both unadjusted and adjusted for a set of common confounders (centre, sex, age, mother's reported asthma and/or hay fever, father's reported asthma and/or hay fever, parental education, maternal smoking during pregnancy, Current environmental smoking at home, Older siblings) comparing the farm children with their reference group. The adjusted odds ratios were significantly lower for current hay fever symptoms, current wheezing, Dr's diagnoses of asthma and atopic sensitisation, e.g. 0.5 (95% CI 0.38-0.65) for current hay fever and 0.53 (95% CI 0.42-0.67) for atopic sensitisation. The odds ratios were also lower for current atopic dermatitis and atopic asthma, but not significantly. Including height and weight in the analyses only lead to minor changes on the above calculated OR's, except for the adjusted OR for atopic asthma which increased from 0.83 to 0.97 (0.57-1.66).

Table 55. Odds ratios comparing the farm children with their reference group.

Odds ratios, farm children compared to their reference group, all children

	Unadjusted		Adjusted*	
	OR	95 % C.I.	OR	95 % C.I.
Current hay fever symptoms	0.4	0.32-0.50	0.50	0.38-0.65
Current wheezing	0.63	0.52-0.77	0.78	0.62-0.98
Doctor's diagnosis of asthma	0.67	0.56-0.80	0.74	0.60-0.92
Current atopic dermatitis	0.72	0.60-0.87	0.83	0.67-1.04
Atopic sensitization	0.55	0.45-0.67	0.53	0.41-0.68
Atopic asthma	0.56	0.36-0.88	0.96	0.57-1.64

* Adjusted for country, sex, age, mother's reported asthma and/or hay fever, father's reported asthma and/or hay fever, parental education, maternal smoking during pregnancy, current environmental smoking at home, older siblings.

In table 56 the same analyses as above are shown but subdivided by country. In Germany the adjusted odds ratios were significantly lower for current hay fever symptoms, current wheezing, Dr's diagnoses of asthma and atopic sensitisation and lower, but not significant for current atopic dermatitis and atopic asthma, when comparing the farm group with their references. In the other countries the results are less consistent. The odds ratios are lower for hay fever and sensitisation in all countries, although not always statistically significant. For current wheezing, and the asthma diagnoses hardly any positive effect is seen from the farming lifestyle. There seems to be a lower risk for current atopic dermatitis in Sweden and Switzerland, comparing the farm children to their references.

Table 56. Odds ratios comparing the farm children with their reference group, subdivided by country.

Odds ratios. farm children compared to their reference group. all children

	Austria		Germany		Holland		Sweden		Switzerland											
	Unadjusted	Adjusted*	Unadjusted	Adjusted*	Unadjusted	Adjusted*	Unadjusted	Adjusted*	Unadjusted	Adjusted*										
	OR	95 % C.I.	OR	95 % C.I.	OR	95 % C.I.	OR	95 % C.I.	OR	95 % C.I.										
Current hay fever symptoms	0.44	0.22-0.87	0.5	0.23-1.12	0.37	0.26-0.53	0.49	0.33-0.73	0.64	0.37-1.10	0.55	0.29-1.04	0.5	0.21-1.17	0.56	0.22-1.45	0.37	0.19-0.70	0.42	0.19-0.93
Current wheezing	5.5	1.25-24	6	1.15-31	0.37	0.26-0.55	0.45	0.30-0.68	1.12	0.77-1.61	1.15	0.75-1.75	0.99	0.43-2.26	1.37	0.55-3.4	0.67	0.40-1.34	0.77	0.41-1.43
Doctor's diagnosis of asthma	0.89	0.40-1.96	0.87	0.34-2.2	0.45	0.32-0.64	0.52	0.36-0.75	1.01	0.73-1.41	1	0.69-1.46	0.81	0.43-1.54	0.96	0.48-1.95	0.8	0.48-1.32	0.83	0.47-1.49
Current atopic dermatitis	0.78	0.40-1.51	0.88	0.40-1.96	0.82	0.61-1.10	0.86	0.62-1.18	1.13	0.82-1.57	1.07	0.73-1.59	0.41	0.16-1.00	0.41	0.16-1.01	0.42	0.19-0.93	0.39	0.16-0.97
Atopic sensitization	0.66	0.41-1.03	0.78	0.47-1.30	0.47	0.32-0.68	0.42	0.27-0.65	0.43	0.23-0.77	0.36	0.17-0.72	0.59	0.32-1.10	0.65	0.33-1.29	0.52	0.33-0.84	0.54	0.29-1.01
Atopic asthma	0.95	0.26-3.4	1.37	0.30-6.4	0.32	0.14-0.73	0.59	0.21-1.62	0.93	0.36-2.4	0.57	0.19-1.72	0.93	0.28-3.1	0.86	0.21-3.6	0.67	0.23-1.95	0.81	0.21-3.26

* Adjusted for country, sex, age, mother's reported asthma and/or hay fever, father's reported asthma and/or hay fever, parental education, maternal smoking during pregnancy, current environmental smoking at home, older siblings.

If dividing the children according to if they are sensitised (IgE > 0.35 kU/l) or not (IgE <= 0.35 kU/l) , the protective effect of farming for asthma and current wheezing appeared stronger among the farm children who were not sensitised than in the sensitised children. This difference is seen in all countries, although most pronounced in Germany and Switzerland (not shown in table).

Many of the questions about the farm life style were only asked to the farm families and the corresponding results can only be presented for this group. In table 57, some of these farm characteristics and some other characteristics are shown. And as can be seen the farm families in the different countries are heterogeneous. The biggest differences seem to be between Switzerland and the other countries. In Switzerland the mothers worked more often on the farm during pregnancy, they use farm milk and wood for cooking to a larger extent. In the Netherlands and Sweden mothers worked to a less extent than in the other countries during pregnancy. The children help more often in the stables and they visit barn more often.

Table 57 Farm characteristics, subdivided by country.

n	All	S	CH	NL	D	A
	2826	331	684	517	966	328
Pets in 1 st year	45.6	71.6	39.4	54.3	38.1	38.4
Mother worked on farm during pregnancy	79.6	68.5	93.4	64.9	80.0	84.3
Farm milk in 1 st year	59.9	40.0	88.0	30.6	54.1	83.3
Farm animal contact in 1 st year	58.5	52.6	70.2	54.4	56.5	52.1
Wood cooking	52.0	16.1	83.0	2.1	63.8	68.2
Daily barn visits	24.9	7.6	48.1	31.0	14.2	16.3
Daily help with hay harvest during season	18.3	15.8	36.0	7.4	11.6	20.4
Bigger farm (>2 employed adults)	36.3	12.4	48.4	11.4	49.6	53.4
Farm						
Former	19.1	29.3	7.9	31.5	19.6	11.0
Part time	23.5	19.9	17.5	8.7	31.0	40.6
Full time	57.5	50.8	74.6	59.8	49.5	48.5
Farm house and stable connected	44.6	0.0	55.6	59.0	31.0	83.1
Cows	69.7	59.2	96.6	57.3	53.5	91.2
Pigs	32.9	15.4	55.3	30.4	28.2	21.7
Poultry	45.7	35.4	47.8	35.2	50.0	55.5
Using hay as animal feed	80.8	74.6	99.7	54.2	78.2	97.0
Using silage as animal feed	53.0	63.1	48.3	59.4	64.6	8.2
Child helps in stable						
Never	15.4	22.1	3.5	20.9	20.2	12.1
Weekly	69.9	71.0	71.0	67.2	66.7	79.0
Daily	14.8	6.9	25.4	11.9	13.1	9.0

The possible impact of the different farming characteristics on allergic diseases, symptoms and atopy are assessed in figure 4 and 5.

Figure 4. Odds ratios for wheezing, coughing, asthma, comparing farm children with and without different characteristics.

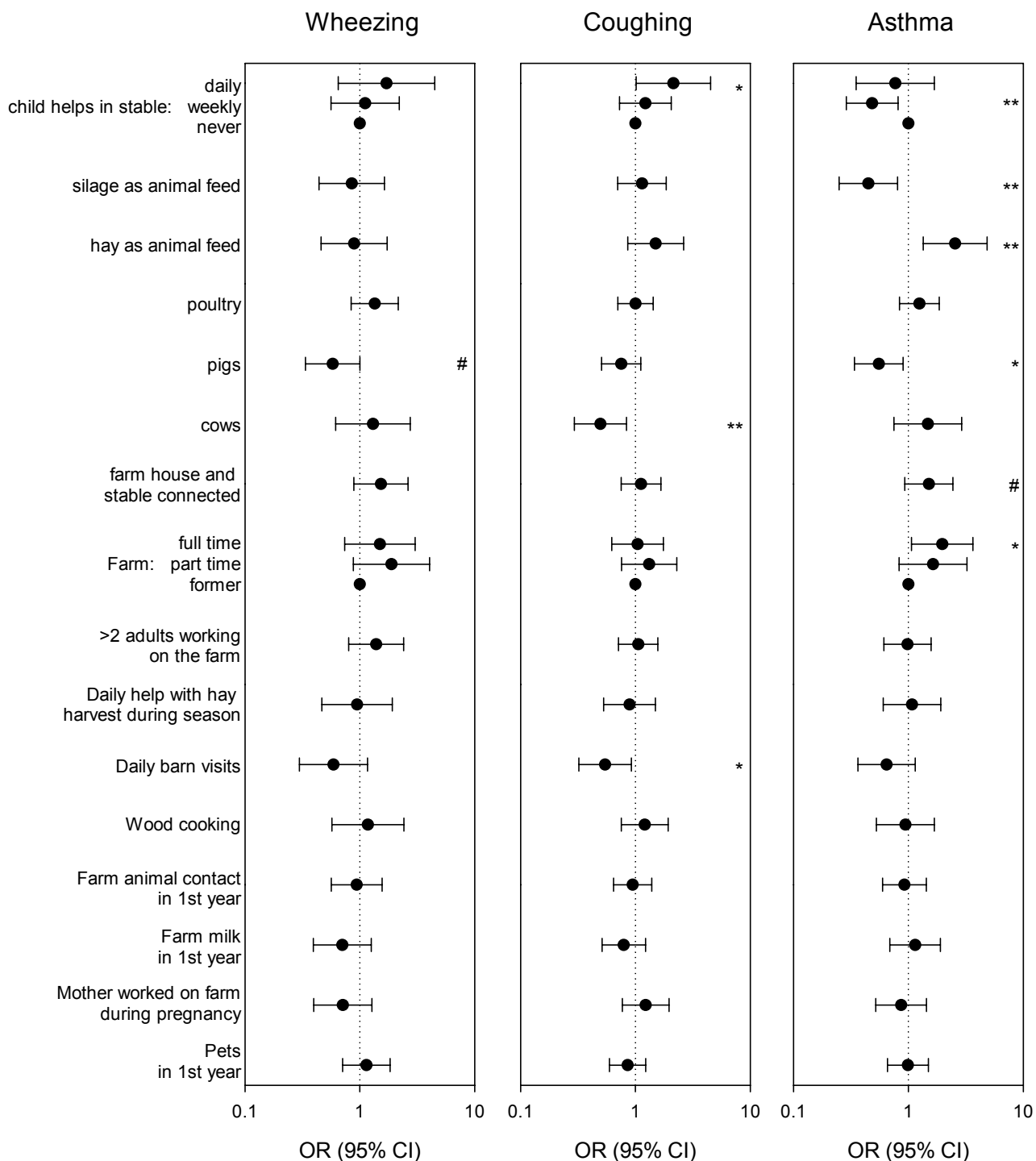
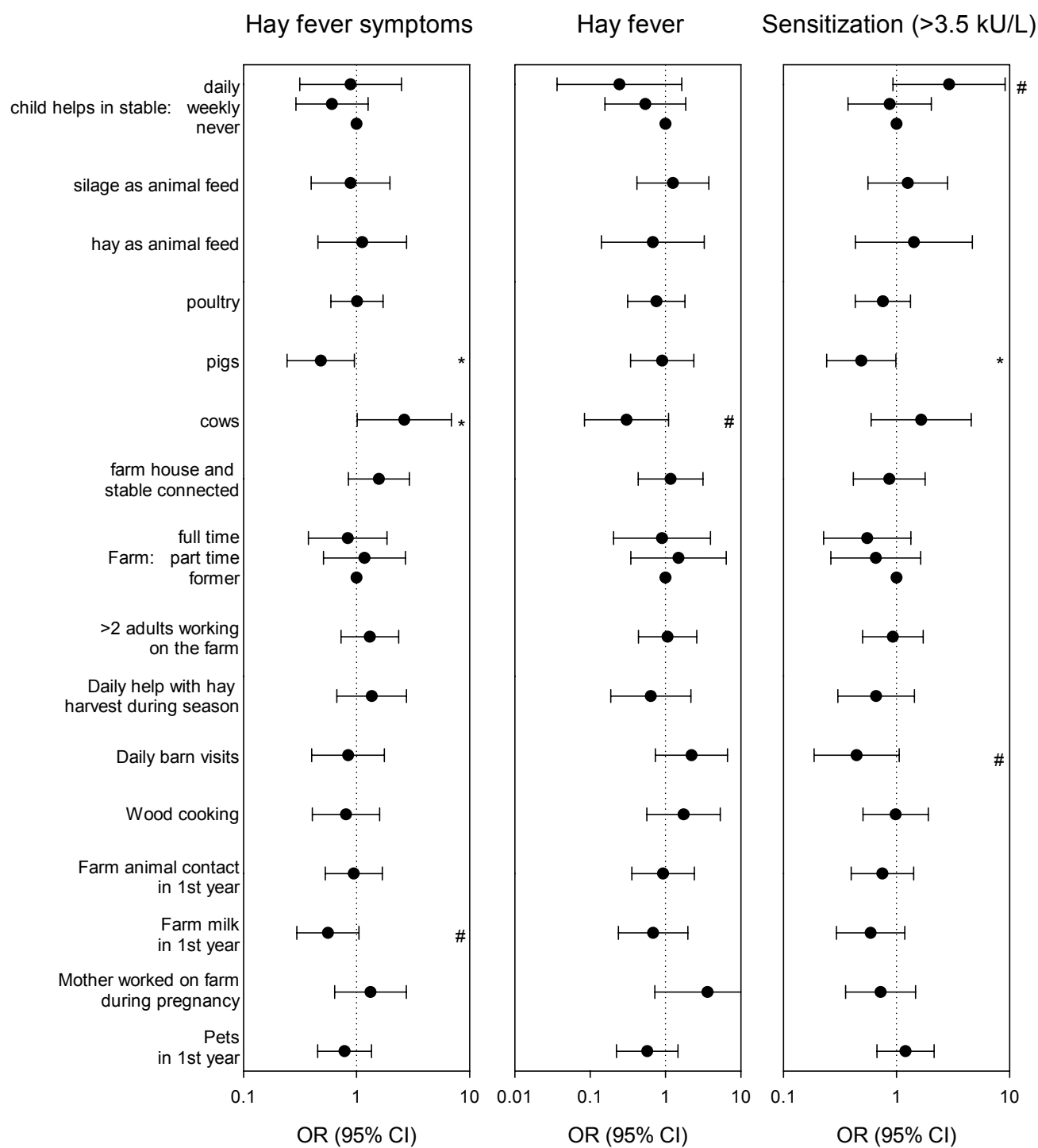


Figure 5. Odds ratios for hay fever symptoms, hay fever and sensitisation (>3.5 kU/L) comparing farm children with and without different characteristics.



It appears that children from farms with pigs and farm children who have daily visits to the barn or who consumed farm milk during the first year experience lower risks of many of the studied health outcomes. Few of the other farming characteristics show consistent associations. Using silage as animal feeds and having pigs appeared as protective factors for asthma. Further analyses of the possible factors behind the protective effect of farming on the risk of development of allergic disease and sensitisation are under way.

Anthroposophic analyses

To study if the degree of anthroposophic lifestyle in the family was of importance for the development of atopic diseases, the Steiner school children were divided in two groups; children with more anthroposophic lifestyle and children with less anthroposophic lifestyle. The parents of children with more anthroposophic lifestyle answered “yes” to the following questions: Did you choose a Steiner school for your child because of the learning method? Did you have an interest in anthroposophy before the child was born? Do you (or anyone of the child’s parents) have an anthroposophic view of life?

There were considerable differences between children with more anthroposophic lifestyle and less anthroposophic lifestyle when looking at anthroposophic characteristics, especially when comparing the prevalence of MMR vaccination having had measles and consumption of a diet based on organic/biodynamic foods (Table 58). Other factors also separated the groups; “having older siblings” was the prominent one with a prevalence of 67% in children with more anthroposophic lifestyle compared to 54% in children with less anthroposophic lifestyle .

Table 58. Distribution of demographic data, factors related to an anthroposophic life style and other potential risk factors for allergic disease in children from Steiner schools and reference schools.

Characteristics	Children with <i>mAL</i> ¹ No (%) (n=2,336)	Children with <i>IAL</i> ¹ No (%) (n=2,271)	Steiner school children ¹ No (%) (n=4,606)	Reference children ¹ No (%) (n=2,024)
Age (years)				
5--6	213 (9)	209 (9)	422 (9)	195 (10)
7--8	739 (32)	662 (29)	1,400 (30)	723 (36)
9	382 (16)	365 (16)	747 (16)	378 (19)
10--11	753 (32)	788 (35)	1,541 (33)	597 (29)
12--13	249 (11)	247 (11)	496 (11)	131 (6)
male/female	1,118/1,218	1,091/1,179	2,209/2,397	995/1,029
Factors related to anthroposophic lifestyle				
Antibiotics first use after 1 year old	819 (35)	932 (41)	1,751 (38)	974 (48)
Antibiotics first use 0-12 months old	302 (13)	486 (21)	788 (17)	623 (31)
Antipyretics first use after 1 year old	693 (30)	847 (37)	1,540 (33)	646 (32)
Antipyretics first use 0-12 months old	297 (13)	626 (28)	923 (20)	1,072 (53)
MMR vaccination	266 (11)	931 (41)	1,197 (26)	1,465 (72)
Child have had measles	994 (43)	542 (24)	1,536 (33)	208 (10)
Fermented vegetables ²	837 (36)	664 (29)	1,501 (33)	335 (17)
Organic/biodynamic food ³	2,000 (86)	1,523 (67)	3,523 (76)	400 (20)
Other factors				
Maternal smoking during pregnancy	162 (7)	204 (9)	366 (8)	277 (14)
Mother reported asthma or hay fever	617 (26)	610 (27)	1,227 (27)	481 (24)
Father reported asthma or hay fever	617 (26)	547 (24)	1,164 (25)	470 (23)
Contact with farm animals 1st year of life	487 (21)	335 (15)	822 (18)	208 (10)
Older siblings	1,557 (67)	1,237 (54)	2,794 (61)	1,038 (51)
Parents education				
Gymnasium etc	683 (29)	719 (32)	1,402 (30)	925 (46)
University etc	1,541 (66)	1,444 (64)	2,985 (65)	766 (38)
Current environmental smoking	288 (12)	342 (15)	630 (14)	545 (27)
Household pets during 1 st year of life	889 (38)	798 (35)	1,687 (37)	571 (28)

¹Children with *mAL* = A Steiner school pupil whose family has reported an anthroposophic view of life.

Children with *IAL* = A Steiner school pupil whose family has not reported an anthroposophic view of life.

Steiner school children = A Steiner school pupil (children with *mAL*+ children with *IAL*).

As shown earlier in the report (table 35-40) the prevalence of most of the allergic diseases as well as atopic sensitisation was lower among the Steiner school children than in their reference group. Table 59 shows data separately for the different countries. The Steiner children with a *more anthroposophic lifestyle* (=mAL) tend to show slightly lower prevalences of the studied health outcomes than the Steiner children with a *less anthroposophic lifestyle* (=IAL). The most pronounced differences are seen for the Swedish children. It should be noted that there are major differences in the prevalence of the health outcomes under study between the countries.

Table 59. Crude prevalence of health outcomes in children with mAL, children with LAL, Steiner school children and reference children.

Health outcomes	Children with mAL		Children with LAL		Steiner school children		Reference children	
	No	(%)	No	(%)	No	(%)	No	(%)
All countries								
Doctor's diagnosis of asthma	197	(8)	224	(10)	421	(9)	217	(11)
Current atopic eczema	180	(8)	212	(9)	392	(9)	211	(10)
Current hay fever symptoms	154	(7)	209	(9)	363	(8)	212	(10)
Current wheezing	193	(8)	205	(9)	398	(9)	168	(8)
Atopic sensitization	246	(33)	141	(32)	387	(32)	248	(39)
Atopic asthma	37	(5)	31	(7)	68	(6)	51	(8)
Sweden								
Doctor's diagnosis of asthma	12	(5)	16	(9)	28	(6)	19	(12)
Current atopic eczema	18	(7)	15	(8)	33	(7)	16	(10)
Current hay fever symptoms	15	(6)	19	(11)	34	(8)	15	(9)
Current wheezing	20	(8)	18	(10)	38	(9)	17	(10)
Atopic sensitization	42	(19)	31	(23)	73	(21)	42	(35)
Atopic asthma	9	(4)	5	(4)	14	(4)	11	(9)
Switzerland								
Doctor's diagnosis of asthma	50	(9)	39	(11)	89	(10)	27	(7)
Current atopic eczema	20	(4)	15	(4)	35	(4)	20	(5)
Current hay fever symptoms	34	(6)	31	(9)	65	(7)	31	(8)
Current wheezing	53	(10)	32	(9)	85	(9)	21	(5)
Atopic sensitization	52	(37)	27	(33)	79	(35)	48	(44)
Atopic asthma	9	(6)	7	(9)	16	(7)	9	(8)
Holland								
Doctor's diagnosis of asthma	26	(6)	61	(10)	87	(9)	67	(16)
Current atopic eczema	58	(14)	89	(15)	147	(14)	71	(17)
Current hay fever symptoms	14	(3)	35	(6)	49	(5)	38	(9)
Current wheezing	32	(7)	55	(9)	87	(9)	58	(14)
Atopic sensitization	30	(32)	20	(29)	50	(31)	38	(36)
Atopic asthma	4	(4)	5	(7)	9	(6)	14	(13)
Germany								
Doctor's diagnosis of asthma	94	(10)	88	(10)	182	(10)	86	(11)
Current atopic eczema	71	(8)	72	(8)	143	(8)	93	(12)
Current hay fever symptoms	79	(9)	99	(11)	178	(10)	109	(14)
Current wheezing	73	(8)	82	(9)	155	(9)	53	(7)
Atopic sensitization	86	(40)	9	(39)	95	(40)	57	(46)
Atopic asthma	13	(6)	2	(9)	15	(6)	8	(6)
Austria								
Doctor's diagnosis of asthma	15	(8)	20	(8)	35	(8)	18	(7)
Current atopic eczema	13	(7)	21	(8)	34	(8)	11	(4)
Current hay fever symptoms	12	(7)	25	(9)	37	(8)	19	(7)
Current wheezing	15	(8)	18	(7)	33	(7)	19	(7)
Atopic sensitization	36	(38)	54	(40)	90	(39)	63	(37)
Atopic asthma	2	(2)	12	(9)	14	(6)	9	(5)

In table 60 the unadjusted and adjusted (centre, sex, age, mother's reported asthma and/or hay fever, father's reported asthma and/or hay fever, parental education, maternal smoking during pregnancy, current environmental smoking at home, Older siblings) odds ratios for atopic diseases and sensitisation are shown comparing the whole Steiner group with their reference group, as well as the children with *the more anthroposophic life style* and *the less anthroposophic lifestyle*. The adjusted odds ratios were significantly lower for current atopic eczema, current hay fever symptoms, and atopic sensitisation, when comparing the whole Steiner group with their references, e.g. the adjusted OR for current hay fever was 0.7 (95% CI 0.57-0.88) and the OR for atopic sensitisation was 0.75 (95% CI 0.59-0.95). The odds ratios were of borderline significance for Dr's diagnoses of asthma and also lower for and atopic asthma, but not significant. Further, the children with *the more anthroposophic life style* tended to have slightly lower odds ratios than the children with *the less anthroposophic life style*.

The risk of different health outcomes associated with certain factors in the anthroposophic life style is presented in 61. In the final models we included a set of variables that are of importance in analyses of childhood allergy and might be potential confounders. Accordingly, in the first model adjusting was done for age, sex and centre (table 61), in model 2 in addition; mothers smoking during pregnancy, mother have reported asthma or hay fever, father have reported asthma or hay fever, contact with farm animals during the child's 1st year of life, current environmental smoking, having older siblings, parents education and household pets. Breastfeeding was included primarily as a potential confounder, but had no significant effect, therefore it was not integrated in the final models. In model 3 we adjusted for the same variables as in model 2, in addition certain factors associated with an anthroposophic lifestyle (see above). Model 4 is similar to model 3, except for the exclusion of antibiotics and antipyretics.

In model 1, we observed a positive association between the use of antibiotics both when introduced during 1st year of life and after 12 months of age, and all outcomes studied, except atopic sensitization. With further adjustment (model 3), this association was valid for asthma, atopic eczema and current wheezing when antibiotics were introduced in 1st year of life and for atopic eczema also when antibiotics was introduced after first year of age.

There was a positive association between the use of antipyretics, both when introduced during 1st year of life and after 12 months of age, and atopic eczema and hay fever symptoms. Diagnosis of asthma was related to first use of antipyretics during 1st year of life, remaining significant also after adjusting for several factors in model 3.

In model 1 there was a significant inverse relation between having symptoms of hay fever and the fact that the child had had measles. This association, however, did not maintain significant when adjustments were made for additional factors (model 2-4). There was no significant risk of any of the selected atopic diseases, in any model, associated with consumption of fermented vegetables.

With varying degrees of adjustment, an inverse association was found between having a diet based on organic/biodynamic food and the risk of atopic eczema and hay fever symptoms. This relation had a borderline significance for both doctor's diagnosis of asthma and atopic asthma in the first model which then disappeared when adjustment were made for possible confounders and factors related to an anthroposophic lifestyle.

Table 60. Odds ratios comparing the Steiner school children and the two different groups of Steiner children (*mAL*= more anthroposophic life style, *LAL*=less anthroposophic lifestyle) with the reference group .

	Children with mAL vs reference children				Children with lAL vs reference children				Steiner school children vs reference children			
	Crude		Adjusted		Crude		Adjusted		Crude		Adjusted	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Dr's diagnosis of asthma	0.77	0.63-0.95	0.79	0.62-1.00	0.88	0.72-1.07	0.86	0.69-1.08	0.82	0.69-0.98	0.84	0.69-1.02
Current atopic eczema	0.73	0.59-0.90	0.67	0.52-0.86	0.82	0.67-1.01	0.80	0.64-1.00	0.78	0.65-0.94	0.76	0.62-0.93
Current hay fever symptoms	0.57	0.46-0.72	0.56	0.43-0.73	0.82	0.67-1.01	0.76	0.60-0.96	0.70	0.58-0.83	0.71	0.57-0.88
Wheezing	1.02	0.82-1.27	1.03	0.80-1.34	1.09	0.88-1.35	1.17	0.92-1.49	1.06	0.88-1.28	1.13	0.91-1.40
Atopy ³	0.73	0.58-0.91	0.77	0.59-1.01	0.75	0.57-0.97	0.67	0.50-0.91	0.74	0.61-0.91	0.75	0.59-0.95
Atopic asthma ³	0.54	0.36-0.81	0.73	0.44-1.21	0.81	0.51-1.27	0.88	0.51-1.50	0.65	0.46-0.92	0.84	0.54-1.29

¹The analyses are conducted among children, age 5-13, from Steiner schools with more or less anthroposophic life style compared to reference children.

²From a logistic regression model adjusting for age, sex and centre (crude), mothers smoking during pregnancy, mother has reported asthma or hay fever, father has reported asthma or hay fever, older siblings, parental education, contact with farm animal during first year of life and household pets during first year of life (adjusted).

³All children included in these analyses have left a blood sample.

Table 61. Odds ratios (OR) for allergic diseases associated with use of antibiotics and antipyretics. The analyses are conducted among Steiner school children and reference children, aged 5-13 years.

	Model 1 ¹		Model 2 ²		Model 3 ³		
Use of antibiotics	<i>No (ref)</i>	<i>First time at age > 12 months</i>	<i>First time at age 0-12 months</i>	<i>First time at age > 12 months</i>	<i>First time at age 0-12 months</i>	<i>First time at age > 12 months</i>	<i>First time at age 0-12 months</i>
<i>Health outcomes</i>							
Doctor's diagnosis of asthma	1.0	2.0 (1.6-2.5)	3.6 (2.8-4.5)	1.5 (1.1-2.1)	3.0 (2.1-4.3)	1.4 (1.0-2.0)	2.5 (1.6-3.8)
Current atopic eczema	1.0	1.5 (1.2-1.9)	1.9 (1.5-2.3)	1.5 (1.1-2.0)	2.0 (1.4-2.8)	1.5 (1.1-2.2)	2.0 (1.3-3.0)
Current hay fever symptoms	1.0	1.6 (1.3-2.0)	1.8 (1.4-2.3)	1.3 (0.9-1.8)	1.6 (1.1-2.3)	1.0 (0.7-1.4)	1.3 (0.8-2.0)
Current wheezing	1.0	1.6 (1.2-1.9)	2.1 (1.6-2.6)	1.2 (0.9-1.7)	1.7 (1.2-2.4)	1.2 (0.8-1.7)	1.8 (1.2-2.8)
Atopy >0.35 kU/L ⁴	1.0	1.3 (1.0-1.6)	1.1 (0.8-1.4)	1.0 (0.7-1.4)	0.9 (0.6-1.3)	1.0 (0.7-1.4)	0.8 (0.5-1.3)
Atopic asthma ⁴	1.0	1.9 (1.1-3.2)	3.0 (1.7-5.1)	1.4 (0.7-3.0)	3.1 (1.4-6.8)	1.2 (0.5-2.6)	2.2 (0.8-5.9)
Use of antipyretics							
<i>Health outcomes</i>							
Doctor's diagnosis of asthma	1.0	1.2 (1.0-1.5)	2.0 (1.6-2.5)	1.1 (0.8-1.5)	1.9 (1.4-2.7)	1.0 (0.7-1.5)	1.6 (1.1-2.4)
Current atopic eczema	1.0	1.5 (1.2-1.9)	1.7 (1.3-2.1)	1.4 (1.0-2.0)	1.8 (1.3-2.5)	1.4 (0.9-2.0)	1.3 (0.8-2.0)
Current hay fever symptoms	1.0	1.5 (1.2-1.8)	1.6 (1.3-2.0)	1.5 (1.1-2.1)	1.4 (1.0-2.0)	1.2 (0.8-1.8)	1.3 (0.8-2.0)
Current wheezing	1.0	1.0 (0.8-1.3)	1.2 (1.0-1.5)	0.9 (0.7-1.3)	1.1 (0.8-1.6)	0.9 (0.6-1.3)	0.9 (0.6-1.4)
Atopy ≥0.35 kU/L ⁴	1.0	1.2 (0.9-1.5)	1.1 (0.8-1.4)	0.9 (0.7-1.3)	1.0 (0.7-1.4)	1.0 (0.6-1.4)	1.1 (0.7-1.8)
Atopic asthma ⁴	1.0	1.0 (0.6-1.7)	1.5 (1.0-2.5)	1.0 (0.5-2.0)	1.6 (0.8-3.1)	0.7 (0.3-1.5)	1.1 (0.5-2.7)

¹From a logistic regression model; adjusted for age, sex, centre.

²From a logistic regression model; adjusted for age, sex, centre and confounders (mothers smoking during pregnancy, mother has reported asthma or hay fever, father has reported asthma or hay fever, contact with farm animals, during 1st year of life, older siblings, parents education, current environmental smoking and household pets during 1st year of life).

³From a logistic regression model; adjusted for age, sex, centre, confounders and factors related to an anthroposophic life style (use of antibiotics and antipyretics, having had the measles, consumption of fermented vegetables and organic/biodynamic food).

⁴All children included in these analyses have left a blood sample.

Table 62. Odds ratios for allergic diseases associated with child having had measles, consumption of fermented vegetables and eating a diet based on organic and biodynamic foods. These analyses are conducted among Steiner school children and reference children, aged 5-13 years.

		Model 1 ¹	Model 2 ²	Model 3 ³	Model 4 ⁴
Child had had measles					
<i>Allergic diseases</i>	<i>No (ref)</i>	<i>Yes</i>	<i>Yes</i>	<i>Yes</i>	<i>Yes</i>
Doctor's diagnosis of asthma	1.0	1.0 (0.8-1.2)	0.8 (0.6-1.1)	1.0 (0.7-1.3)	0.8 (0.6-1.1)
Current atopic eczema	1.0	1.0 (0.8-1.3)	1.1 (0.8-1.5)	1.4 (1.0-1.9)	1.2 (0.9-1.7)
Current hay fever symptoms	1.0	0.7 (0.6-0.9)	0.9 (0.6-1.2)	1.0 (0.7-1.5)	1.0 (0.7-1.4)
Current wheezing	1.0	1.2 (1.0-1.5)	1.2 (0.9-1.7)	1.4 (1.0-1.9)	1.3 (0.9-1.8)
Atopy ≥ 0.35 kU/L ⁵	1.0	0.8 (0.7-1.0)	1.0 (0.7-1.3)	0.9 (0.7-1.3)	1.0 (0.7-1.3)
Atopic asthma ⁵	1.0	0.7 (0.4-1.1)	0.5 (0.2-1.0)	0.5 (0.2-1.1)	0.5 (0.2-1.1)
Consumption of fermented vegetables⁶					
<i>Allergic diseases</i>	<i>No (ref)</i>				
Doctor's diagnosis of asthma	1.0	0.9 (0.8-1.1)	0.8 (0.6-1.1)	0.9 (0.6-1.2)	0.8 (0.6-1.2)
Current atopic eczema	1.0	1.0 (0.8-1.2)	0.9 (0.7-1.2)	1.0 (0.7-1.4)	1.0 (0.7-1.3)
Current hay fever symptoms	1.0	0.9 (0.8-1.2)	0.8 (0.6-1.1)	0.95 (0.7-1.4)	0.9 (0.6-1.2)
Current wheezing	1.0	0.9 (0.8-1.2)	0.8 (0.6-1.1)	0.9 (0.6-1.2)	0.8 (0.6-1.2)
Atopy ≥ 0.35 kU/L ⁵	1.0	1.0 (0.8-1.3)	1.0 (0.7-1.4)	1.0 (0.7-1.4)	1.0 (0.7-1.4)
Atopic asthma ⁵	1.0	0.7 (0.4-1.1)	0.7 (0.4-1.4)	0.8 (0.4-1.6)	0.8 (0.4-1.7)
Diet based on organic/biodynamic foods⁷					
<i>Allergic diseases</i>	<i>No (ref)</i>				
Doctor's diagnosis of asthma	1.0	0.8 (0.7-0.99)	0.9 (0.7-1.1)	1.2 (0.9-1.7)	1.0 (0.7-1.3)
Current atopic eczema	1.0	0.8 (0.7-0.95)	0.7 (0.6-0.9)	0.7 (0.5-1.0)	0.6 (0.5-0.9)
Current hay fever symptoms	1.0	0.7 (0.6-0.8)	0.7 (0.5-0.95)	0.7 (0.5-0.96)	0.7 (0.5-0.9)
Current wheezing	1.0	0.9 (0.8-1.1)	0.9 (0.7-1.2)	1.0 (0.7-1.4)	0.9 (0.7-1.3)
Atopy ≥ 0.35 kU/L ⁵	1.0	0.9 (0.7-1.0)	1.1 (0.8-1.5)	1.3 (0.9-1.8)	1.2 (0.9-1.7)
Atopic asthma ⁵	1.0	0.7 (0.4-0.96)	0.7 (0.4-1.2)	1.2 (0.6-2.5)	0.9 (0.5-1.7)

¹From a logistic regression model; adjusted for age, sex, country.

²From a logistic regression model; adjusted for age, sex, country and confounders (mothers smoking during pregnancy, mother reported asthma or hay fever, father has reported asthma or hay fever, contact with farm animals, during 1st year of life, older siblings, parents education, current environmental smoking and household pets during 1st year of life).

³From a logistic regression model; adjusted for age, sex, country, confounders and factors related to an anthroposophic life style (use of antibiotics and antipyretics, having had measles, consumption of fermented vegetables and organic/biodynamic food)

⁴From a logistic regression model; adjusted for age, sex, country, confounders, factors related to an anthroposophic life style, EXCEPT antibiotics and antipyretics.

⁵All children included in these analyses have left a blood sample.

⁶Child that eats fermented vegetables regularly during at least one year.

⁷The child's diet has mainly been based on organic and biodynamic food.

As shown above the Steiner school children have a lower prevalence of all allergic diseases studied, except current wheezing. Also, certain allergic diseases were associated with the use of antibiotics, antipyretics and a diet based on organic and biodynamic food. Other studies have also reported lower prevalence of allergy in Steiner school children and associations between these exposures and allergy (Alm et al. 1999; Wickens et al. 1999).

In this study, the more frequent use of antibiotics and antipyretics among reference children compared to Steiner school children are concordant with what Alm et al described (Alm et al. 1999). The same was seen for MMR vaccination. From New Zealand Wickens et al (Wickens et al. 1999) reported that the prevalence of antibiotic use in 1st year of life in Steiner school children was 36%. In PARSIFAL this number is 13% among children with more anthroposophic lifestyle and 21% among children with less anthroposophic lifestyle and might therefore be related to the degree of anthroposophic life style in the family.

Whether measles could have a protective effect on atopy is controversial and study results are contradictory. In PARSIFAL 33% of the Steiner school children that had had measles were atopic and 32% among children who had not had measles (data not shown). Shaheen et al (Shaheen et al. 1996) found lower prevalence of atopy among children in Guinea Bissau who had experienced measles infection. However, Paunio et al concluded (Paunio et al. 2000) that measles and atopy frequently occur together which does not support the hypothesis that measles would protect against atopy.

The prevalence of atopic disease in reference children in Austria, Germany and Sweden can be compared with prevalence reports from the 13-14 years old children in the ISAAC study (1998). The prevalence data for Austria is comparable for eczema (4% vs. 5%) and hay fever (7% vs. 10%). For Germany eczema prevalence appears higher in PARSIFAL (12% vs. 7%) and hay fever symptoms the same (14% vs. 14%). For Sweden the ISAAC study reports higher prevalence of both eczema (10% vs. ~15%) and hay fever (9% vs. 12%). The higher prevalence of hay fever in ISAAC could be explained by a higher age of the children, this may would also explain the lower prevalence of eczema in PARSIFAL.

Concerning antibiotics both positive (Wickens et al. 1999; Cullinan et al. 2004), and negative (Farooqi et al. 1998; Celedon et al. 2002) associations with development of asthma have been reported. The findings could be explained by an influence of the medication on the immune system or by the fact that a child with asthma is given antibiotics on the presumption that it has a respiratory infection. The study by Wickens (Wickens et al. 1999) was conducted among children in Steiner schools in New Zealand and a positive association between antibiotic use and asthma was found, but when adjusted for all potential confounders it became weaker. In PARSIFAL we found a positive association between asthma and use of antibiotics. There was also a positive association between atopic eczema and use of antibiotics, but primarily when antibiotics were used during the child's first year of life.

A diet based on organic and biodynamic foods probably contains lower amounts of toxic substances of human origin. Children who consumed this diet had a lowered risk of atopic eczema and hay fever symptoms. Alm et al (Alm et al. 1999) found a decreased risk for atopy associated with this diet. It has been reported that vegetarian diets might alleviate symptoms of severe atopic eczema (Rosenfeldt et al. 2003). Lindahl et al reported a reduced need of medication in the treatment of bronchial asthma associated with vegetarian diet (Lindahl et al. 1985). The fact that a vegetarian diet improves symptoms of eczema could perhaps contribute to the lower prevalence in Steiner school children. Several studies show that probiotics reduce symptoms of atopic eczema (Kalliomaki et al. 2001; Kirjavainen et al. 2003; Lodinova-Zadnikova

et al. 2003), but there was no association with the consumption of fermented vegetables containing lactobacilli.

The anthroposophic life style factors studied here are just a selection of various characteristics in the anthroposophic life style. Most certainly there are several factors, not included in this study, that need to be taken into consideration before the lower risk of allergic diseases among anthroposophic children is fully understood.

Dietary factors and their relation to asthma and allergy

The analyses above show that farm children and children with an anthroposophic lifestyle have a lower asthma and allergy prevalence. One of the main hypotheses in the study was that the different diets in the different sub-groups could play an important role.

In the following section some preliminary analyses are presented.

As seen in table 63, there are many differences between the farm children, their references and the Steiner school children and their references. Both the farm children and the Steiner school children drink more farm milk, consume more home made butter, eat more home grown vegetables and home produced meat.

Table 63. Do farmers' children or children visiting a Steiner school have characteristic food patterns?

	Farmers' children (versus rural children without farmers' parents) consume:		Steiner school children (versus suburban / urban children visiting a public school) consume:		Full time farmers' children (versus children from a anthroposophic family ⁴) consume:	
	more ¹	less ¹	more ¹	less ¹	more ¹	less ¹
milk						
any type of milk ³ (daily)	x				x	
any type of farm milk (any, daily)	xx		xx		xx	
any type of farm milk 1 st yr ²	xx		xx			
any type of shop milk (any, daily)		xx				xx
full cream shop milk, full cream shop milk 1 st yr		xx	x			xx
low cream shop milk				x	x	
low cream shop milk 1 st yr		xx		xx	x	
UHT shop milk, UHT shop milk 1 st yr		xx		xx	xx	
all other foods						
butter ³ (daily)	x		x			
butter 1 st yr					x	
home-made butter	xx		x		xx	
margarine (daily)				xx	x	
margarine 1 st yr				xx	xx	
home-made yogurts	xx		x		xx	
meat ³ (daily)	x			xx	xx	
home-made meat	xx		x		xx	
meat 1 st yr, fish 1 st yr				xx	xx	
eggs ³ (daily)	xx			xx	xx	
home-made eggs, eggs 1 st yr	x		x		xx	
potatoes ³ (daily)	x			xx	xx	
home-made potatoes	x		x		xx	
vegetables ³ (daily)	x		x			x
home-made vegetables	x		x		x	
fruits ³ (daily)			x			x
home-made fruits	x		x		x	
whole meal products ³ (daily)			xx			xx
home-made whole meal products	x		x		x	
whole meal products ³ 1 st yr			x			xx
olive oil (any, daily), olive oil 1 st yr		x	x			xx
other vegetable oils (daily)			xx			xx
peanuts 1 st yr				x		
nuts 1 st yr	x		xx			x
fermented vegetables ³ (any)			x			x
home-made fermented vegetables	x		xx			
food production						
foods from biologic production			xx			xx
foods from bio-dynamic production		xx	xx			xx
breastfeeding						
breastfeeding ≥ 6 months			x			xx
excl. breastfeeding ≥ 3 months			x			x
excl. breastfeeding ≥ 5 months			x			xx

¹ significant ($p \leq 0.05$) difference of > 2 fold more or less children: xx, 1.3-2 fold more or less children: x

² any consumption in the first year of life

³ home-made and shop combined

⁴ defined as: anthroposophic pedagogy was the most important reason for choosing a RSS, and, at least the mother has a anthroposophic life engagement, and, parents were interested in anthroposophy before the child's birth

As shown in table 64 significantly ($p < 0.001$) more farm and Steiner school children consume home-produced dairy products compared to their reference groups: farm milk (F 64% versus FR 13%; S 16% versus SR 5%), home-produced butter (F 39% versus FR 14%; S 12% versus SR 9%), home-made yoghurt (F 39% versus FR 17%; S 18% versus SR 12%).

In multivariate logistic models adjusted for potential confounders and current farm milk consumption, farm milk consumption before the first birthday was inversely related to diagnosed asthma (OR 0.77; 95% CI 0.62-0.94), hay fever (0.64; 0.49-0.84) and pollen sensitisation (0.76; 0.59-0.97). Current home-produced butter was also inversely related to asthma (0.71; 0.57-0.88) and hay fever (0.79; 0.59-1.06) but not to atopic sensitisation. No significant associations were observed between consumption of meat, vegetables or fruits, and the prevalence of allergy. In conclusion it appears like home produced dairy products may play a role for the reduced occurrence of asthma and allergy in children.

Table 64. Daily farm milk consumption vs. health outcomes. Reference (farm or shop milk consumption less than daily or never)

Health endpoint	Model A	Model B	Model C	Model D	Model E	Model F
	unadjusted	A + BMI + height + age	B + study center + parental history of asthma or hay fever + parents' education	C + study group	C + farm milk consumption 1 st yr	C + study group + farm milk consumption 1 st yr
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	n (%)OR (95% CI)
Dr's diagnoses asthma	0.58 (0.47-0.72)	0.57 (0.45-0.72)	0.68 (0.53-0.87)	0.78 (0.59-1.03)	0.83 (0.63-1.09)	0.89 (0.67-1.19)
Current wheezing	0.47 (0.36-0.60)	0.49 (0.38-0.64)	0.57 (0.43-0.76)	0.66 (0.49-0.90)	0.63 (0.47-0.85)	0.71 (0.52-0.97)
Dr's diagnoses hay fever	0.40 (0.29-0.55)	0.39 (0.28-0.55)	0.53 (0.37-0.76)	0.83 (0.56-1.24)	0.76 (0.52-1.11)	1.03 (0.69-1.55)
Current hay fever symptoms	0.41 (0.31-0.54)	0.39 (0.29-0.52)	0.50 (0.37-0.68)	0.68 (0.49-0.96)	0.69 (0.50-0.96)	0.83 (0.59-1.18)
Current atopic dermatitis	0.44 (0.34-0.56)	0.45 (0.35-0.59)	0.64 (0.48-0.85)	0.69 (0.51-0.94)	0.73 (0.54-1.00)	0.76 (0.55-1.04)
Ever atopic dermatitis symptoms + location	0.54 (0.45-0.66)	0.54 (0.44-0.66)	0.69 (0.56-0.86)	0.75 (0.60-0.95)	0.75 (0.60-0.94)	0.79 (0.62-1.01)
Current atopic dermatitis symptoms	0.52 (0.42-0.64)	0.52 (0.42-0.65)	0.62 (0.49-0.78)	0.67 (0.52-0.87)	0.67 (0.52-0.86)	0.70 (0.54-0.91)
Atopic sensitisation	0.64 (0.53-0.78)	0.63 (0.52-0.77)	0.61 (0.49-0.76)	0.85 (0.66-1.08)	0.67 (0.53-0.86)	0.85 (0.66-1.10)

6.5 Conclusions

A first aim of the study was to examine if the results from the few earlier studies showing lower prevalence of atopic disease among farm and Steiner school children could be confirmed and if there are any differences in the possible protecting effect from these lifestyles between five countries in Europe (Austria, Germany, Holland, Sweden and Switzerland). Overall the results are very clear, there is a difference in prevalence of both subjective and objective markers of atopic disease when comparing the farm children and their reference group as well as when comparing the Steiner school group and their reference group.

The protective effect of farming is found for all of the studied atopy outcomes, both self-reported, as for example current hay fever symptoms, wheezing, atopic eczema and asthma, and objective in the form of sensitisation (serum-IgE ≥ 0.35 kU/l) when studying all countries together. The risk of having current hay fever or being sensitised is only about 50% (OR 0.50 (95 CI 0.38-0.65) and OR 0.53 (95 CI 0.41-0.68), respectively, for the farm children compared to their references. In the German subset the results look quite the same as for all the children, whereas the protective effect among farm children in the other countries appears consistent mostly for hay fever and sensitisation.

The protective effect of the anthroposophic life style is found for all of the studied outcomes except for current wheezing. For example, the OR of having current hay fever or being sensitised is 0.71 (95 CI 0.57-0.88) and 0.75 (95 CI 0.59-0.95), respectively, for the Steiner school children compared to their references. Also here there were some differences between the countries. Hardly any effect from the anthroposophic life style is found in the Austrian sub sample, and in Switzerland a clear lower risk in the Steiner group was seen only for sensitisation. Consistent protective effects of the anthroposophic life style are found primarily in Germany, the Netherlands and Sweden.

A second aim of the study was to assess the possible role of certain environmental and lifestyle factors for the protective effect of the farming and Steiner life style. The analyses are still in progress and the results presented here should be considered preliminary. For the farm children, we found that children living on farms with pigs and farm children who have daily visits to the barn and those who consumed farm milk during the first year or home made dairy products generally have lower risks of the atopy outcomes. Few of the other studied farming characteristics seem to show consistent effects. For the Steiner children there was an increased risk for asthma, atopic eczema and current wheezing associated with first use of antibiotics during 1st year of life. Atopic eczema was also related to the use of antibiotics after 12 months of age. Use of antipyretics in 1st year of life was associated with a higher risk for asthma. Furthermore, consumption of organic/biodynamic food was related to a lower risk for atopic eczema and hay fever symptoms.

The first results from the house dust analyses demonstrate that farm children and - to a lesser extent - Steiner children, are exposed to higher levels of endotoxin, EPS and glucans, than their respective references. This may contribute to the lower prevalence of atopy in these groups. Further analyses studying the relationship between components in the dust samples and atopy are in progress.

One potentially weak point in several studies is that the estimated prevalence of asthma is usually based on questionnaire data. Questionnaires may be differently answered/interpreted by culturally different groups. However, in the PARSIFAL study we found no substantial differences in the association between BHR to hypertonic saline and questionnaire information

on 'asthma' and 'wheeze' among the farm children, Steiner school children, farm reference children and Steiner reference children. This suggests that comparisons of symptoms between the different groups in our study were valid.

Preliminary analyses of the faecal samples suggest differences in the intestinal bacterial flora between Steiner children and their references, as well as a presence of certain bacteria in children from pig farms. Further analyses are under way.

In view of the limited information available on the quality of dietary information for children obtained via questionnaire we investigated validity and reproducibility of the dietary data in PARSIFAL. Overall we found moderate to acceptable validity and moderate to substantial reproducibility in the parental reports of their children's diet. The quality was considered sufficiently accurate for diet-disease analyses of most foods.

In conclusion the PARSIFAL-study confirms that there is a protective effect from a farming and anthroposophic lifestyle for the development of allergy. Extensive ongoing analyses will further study the role of specific environmental and life style factors for this effect.

6.6 Dissemination and exploitation of the results

The dissemination of the results will be through many different ways:

1. The study report to the EU.
2. Publications in scientific journals. Some articles have already been written and sent in for publication and many others will be written. For a more thorough list of the planned articles see “6.7 main literature produced”.
3. At least two PhD-thesis and two master thesis based on the findings from the project will be written.
4. Presentations at professional meetings aimed at the relevant scientific and policy audiences. Three abstracts describing the first results from the PARSIFAL study have already been accepted, one will be presented at EAACI (European Academy of Allergology and Clinical Immunology) and two at the ERS meeting (European Respiratory Society).
5. Collaboration with international and national organisations engaged in allergy prevention.
6. Specially prepared material for mass media and the general public, including press releases and brochures. A first brochure describing the project has already been published and is appended the report. When the results have been thoroughly analysed more material will be produced for mass media and the general public.

In summary the study is expected to provide new insights to the role of certain environmental and life style characteristics for the development of allergy in children, which can contribute to guidelines for prevention of childhood allergy. However, as discussed above, the data analyses are still ongoing and it is too early to give any new recommendations.

6.7 Main literature produced

The PARSIFAL project is in a phase where work on several scientific articles is going on in all the participating centres. The planned and already submitted articles are listed below. Many of the results will also be presented at different scientific meetings, both national and international.

- Prevalence of atopy in children related to farming and anthroposophic life style in five European countries – The PARSIFAL study.
- Atopy in school children from anthroposophic families in five European countries.
- Determinants of gut microflora among farm children, Steiner school children and references.
- Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children. (submitted for publication)
- House dust mite, pet and storage mite allergens in house dust of farm children, Rudolf Steiner school children and references
- Biological components in house dust: A comparison of two dust sampling methods (nylon socks and ALK nozzles)
- Levels of bacterial and fungal components in house dust and wheeze in children.
- The effect of farm living on sensitisation among children in five European countries
- Effects of farm milk and dairy products on asthma/allergy in children.
- Yogurts and other dairy products and atopic dermatitis
- Validity and reproducibility of a food questionnaire administered to parents of 5- to 13-year old school children (submitted for publication).
- Bronchial hyperresponsiveness used to assess the sensitivity and specificity questionnaire response of wheezing and asthma in children.

6.8 References:

- (1997). European Allergy White Paper.
- (1998). "Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee." Lancet **351**(9111): 1225-32.
- Alm, J. S., J. Swartz, B. Björkstén, L. Engstrand, J. Engström, I. Kuhn, et al. (2002). "An anthroposophic lifestyle and intestinal microflora in infancy." Pediatr Allergy Immunol **13**(6): 402-11.
- Alm, J. S., J. Swartz, G. Lilja, A. Scheynius and G. Pershagen (1999). "Atopy in children of families with an anthroposophic lifestyle." Lancet **353**(9163): 1485-8.
- Arnold, J. E., T. Rohan, G. Howe and M. Leblanc (1995). "Reproducibility and validity of a food-frequency questionnaire designed for use in girls age 7 to 12 years." Ann Epidemiol **5**(5): 369-77.
- Baranowski, T., D. Sprague, J. H. Baranowski and J. A. Harrison (1991). "Accuracy of maternal dietary recall for preschool children." J Am Diet Assoc **91**(6): 669-74.
- Bischof, W., A. Koch, U. Gehring, B. Fahlbusch, H. E. Wichmann and J. Heinrich (2002). "Predictors of high endotoxin concentrations in the settled dust of German homes." Indoor Air **12**(1): 2-9.
- Björkstén, B. (1999). "The intrauterine and postnatal environments." J Allergy Clin Immunol **104**(6): 1119-27.
- Bråbäck, L., A. Breborowicz, S. Dreborg, A. Knutsson, H. Pieklik and B. Björkstén (1994). "Atopic sensitization and respiratory symptoms among Polish and Swedish school children." Clin Exp Allergy **24**(9): 826-35.
- Braun-Fahrlander, C., M. Gassner, L. Grize, U. Neu, F. H. Sennhauser, H. S. Varonier, et al. (1999). "Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution." Clin Exp Allergy **29**(1): 28-34.
- Braun-Fahrlander, C., J. Riedler, U. Herz, W. Eder, M. Waser, L. Grize, et al. (2002). "Environmental exposure to endotoxin and its relation to asthma in school-age children." N Engl J Med **347**(12): 869-77.
- Brismar, B., C. Edlund and C. E. Nord (1993). "Impact of cefpodoxime proxetil and amoxicillin on the normal oral and intestinal microflora." Eur J Clin Microbiol Infect Dis **12**(9): 714-9.
- Burney, P. G., S. Chinn and R. J. Rona (1990). "Has the prevalence of asthma increased in children? Evidence from the national study of health and growth 1973-86." Bmj **300**(6735): 1306-10.
- Byers, T., F. Trieber, E. Gunter, R. Coates, A. Sowell, S. Leonard, et al. (1993). "The accuracy of parental reports of their children's intake of fruits and vegetables: validation of a food frequency questionnaire with serum levels of carotenoids and vitamins C, A, and E." Epidemiology **4**(4): 350-5.
- Celedon, J. C., A. A. Litonjua, L. Ryan, S. T. Weiss and D. R. Gold (2002). "Lack of association between antibiotic use in the first year of life and asthma, allergic rhinitis, or eczema at age 5 years." Am J Respir Crit Care Med **166**(1): 72-5.
- Chew, G. L., J. Douwes, G. Doekes, K. M. Higgins, R. van Strien, J. Spithoven, et al. (2001). "Fungal extracellular polysaccharides, beta (1-->3)-glucans and culturable fungi in repeated sampling of house dust." Indoor Air **11**(3): 171-8.

- Cullinan, P., J. Harris, P. Mills, S. Moffat, C. White, J. Figg, et al. (2004). "Early prescriptions of antibiotics and the risk of allergic disease in adults: a cohort study." Thorax **59**(1): 11-5.
- Douwes, J., B. van der Sluis, G. Doekes, F. van Leusden, L. Wijnands, R. van Strien, et al. (1999). "Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms." J Allergy Clin Immunol **103**(3 Pt 1): 494-500.
- Eck, L. H., R. C. Klesges and C. L. Hanson (1989). "Recall of a child's intake from one meal: are parents accurate?" J Am Diet Assoc **89**(6): 784-9.
- Farooqi, I. S. and J. M. Hopkin (1998). "Early childhood infection and atopic disorder." Thorax **53**(11): 927-32.
- Heinrich, J., B. Holscher, J. Douwes, K. Richter, A. Koch, W. Bischof, et al. (2003). "Reproducibility of allergen, endotoxin and fungi measurements in the indoor environment." J Expo Anal Environ Epidemiol **13**(2): 152-60.
- Hinze, S., K. C. Bergmann, H. Löwenstein and G. N. Hansen (1997). "Cow hair allergen (Bos d 2) content in house dust: correlation with sensitization in farmers with cow hair asthma." Int Arch Allergy Immunol **112**(3): 231-7.
- Iversen, M., J. Korsgaard, T. Hallas and R. Dahl (1990). "Mite allergy and exposure to storage mites and house dust mites in farmers." Clin Exp Allergy **20**(2): 211-9.
- Kalliomaki, M., S. Salminen, H. Arvilommi, P. Kero, P. Koskinen and E. Isolauri (2001). "Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial." Lancet **357**(9262): 1076-9.
- Kirjavainen, P. V., S. J. Salminen and E. Isolauri (2003). "Probiotic bacteria in the management of atopic disease: underscoring the importance of viability." J Pediatr Gastroenterol Nutr **36**(2): 223-7.
- Kjellman, N. I. (1994). "Natural course of asthma and allergy in childhood." Pediatr Allergy Immunol **5**(6 Suppl): 13-8.
- Klesges, R. C., L. M. Klesges, G. Brown and G. C. Frank (1987). "Validation of the 24-hour dietary recall in preschool children." J Am Diet Assoc **87**(10): 1383-5.
- Klintberg, B., N. Berglund, G. Lilja, M. Wickman and M. van Hage-Hamsten (2001). "Fewer allergic respiratory disorders among farmers' children in a closed birth cohort from Sweden." Eur Respir J **17**(6): 1151-7.
- Landis, J. R. and G. G. Koch (1977). "The measurement of observer agreement for categorical data." Biometrics **33**(1): 159-74.
- Lindahl, O., L. Lindwall, A. Spångberg, A. Stenram and P. A. Öckerman (1985). "Vegan regimen with reduced medication in the treatment of bronchial asthma." J Asthma **22**(1): 45-55.
- Livingstone, M. B., A. M. Prentice, W. A. Coward, J. J. Strain, A. E. Black, P. S. Davies, et al. (1992). "Validation of estimates of energy intake by weighed dietary record and diet history in children and adolescents." Am J Clin Nutr **56**(1): 29-35.
- Livingstone, M. B. and P. J. Robson (2000). "Measurement of dietary intake in children." Proc Nutr Soc **59**(2): 279-93.
- Lodinova-Zadnikova, R., B. Cukrowska and H. Tlaskalova-Hogenova (2003). "Oral administration of probiotic Escherichia coli after birth reduces frequency of allergies and repeated infections later in life (after 10 and 20 years)." Int Arch Allergy Immunol **131**(3): 209-11.
- Paunio, M., O. P. Heinonen, M. Virtanen, P. Leinikki, A. Patja and H. Peltola (2000). "Measles history and atopic diseases: a population-based cross-sectional study." Jama **283**(3): 343-6.

- Potischman, N., H. A. Weiss, C. A. Swanson, R. J. Coates, M. D. Gammon, K. E. Malone, et al. (1998). "Diet during adolescence and risk of breast cancer among young women." J Natl Cancer Inst **90**(3): 226-33.
- Radon, K., B. Danuser, M. Iversen, E. Monso, C. Weber, J. Hartung, et al. (2002). "Air contaminants in different European farming environments." Ann Agric Environ Med **9**(1): 41-8.
- Radon, K., A. Schottky, S. Garz, F. Koops, D. Szadkowski, D. Nowak, et al. (2000). "Distribution of dust-mite allergens (Lep d 2, Der p 1, Der f 1, Der 2) in pig-farming environments and sensitization of the respective farmers." Allergy **55**(3): 219-25.
- Riedler, J., C. Braun-Fahrländer, W. Eder, M. Schreuer, M. Waser, S. Maisch, et al. (2001). "Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey." Lancet **358**(9288): 1129-33.
- Riedler, J., W. Eder, G. Oberfeld and M. Schreuer (2000). "Austrian children living on a farm have less hay fever, asthma and allergic sensitization." Clin Exp Allergy **30**(2): 194-200.
- Rockett, H. R., C. S. Berkey and G. A. Colditz (2003). "Evaluation of dietary assessment instruments in adolescents." Curr Opin Clin Nutr Metab Care **6**(5): 557-62.
- Rosenfeldt, V., E. Benfeldt, S. D. Nielsen, K. F. Michaelsen, D. L. Jeppesen, N. H. Valerius, et al. (2003). "Effect of probiotic Lactobacillus strains in children with atopic dermatitis." J Allergy Clin Immunol **111**(2): 389-95.
- Shaheen, S. O., P. Aaby, A. J. Hall, D. J. Barker, C. B. Heyes, A. W. Shiell, et al. (1996). "Measles and atopy in Guinea-Bissau." Lancet **347**(9018): 1792-6.
- Shea, S., C. E. Basch, M. Irigoyen, P. Zybert, J. L. Rips, I. Contento, et al. (1991). "Relationships of dietary fat consumption to serum total and low-density lipoprotein cholesterol in hispanic preschool children." Prev Med **20**(2): 237-49.
- Stein, A. D., S. Shea, C. E. Basch, I. R. Contento and P. Zybert (1992). "Consistency of the Willett semiquantitative food frequency questionnaire and 24-hour dietary recalls in estimating nutrient intakes of preschool children." Am J Epidemiol **135**(6): 667-77.
- Sudo, N., S. Sawamura, K. Tanaka, Y. Aiba, C. Kubo and Y. Koga (1997). "The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction." J Immunol **159**(4): 1739-45.
- Treiber, F. A., S. B. Leonard, G. Frank, L. Musante, H. Davis, W. B. Strong, et al. (1990). "Dietary assessment instruments for preschool children: reliability of parental responses to the 24-hour recall and a food frequency questionnaire." J Am Diet Assoc **90**(6): 814-20.
- van Strien, R. T., L. P. Koopman, M. Kerkhof, J. Spithoven, J. C. de Jongste, J. Gerritsen, et al. (2002). "Mite and pet allergen levels in homes of children born to allergic and nonallergic parents: the PIAMA study." Environ Health Perspect **110**(11): A693-8.
- Wickens, K., N. Pearce, J. Crane and R. Beasley (1999). "Antibiotic use in early childhood and the development of asthma." Clin Exp Allergy **29**(6): 766-71.
- Wickman, M., I. Kull, G. Pershagen and S. L. Nordvall (2002). "The BAMSE project: presentation of a prospective longitudinal birth cohort study." Pediatr Allergy Immunol **13 Suppl 15**: 11-3.
- Willet, W. (1998). Nutritional Epidemiology 2nd ed. New York, Oxford University Pres.
- Vobecky, J. S., J. Vobecky and S. Froda (1988). "The reliability of the maternal memory in a retrospective assessment of nutritional status." J Clin Epidemiol **41**(3): 261-5.
- Wold, A. E. (1998). "The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora?" Allergy **53**(46 Suppl): 20-5.

- Von Ehrenstein, O. S., E. Von Mutius, S. Illi, L. Baumann, O. Böhm and R. von Kries (2000). "Reduced risk of hay fever and asthma among children of farmers." Clin Exp Allergy **30**(2): 187-93.
- Von Mutius, E., C. Braun-Fahrlander, R. Schierl, J. Riedler, S. Ehlermann, S. Maisch, et al. (2000). "Exposure to endotoxin or other bacterial components might protect against the development of atopy." Clin Exp Allergy **30**(9): 1230-4.
- Wouters, I. M., J. Douwes, G. Doekes, P. S. Thorne, B. Brunekreef and D. J. Heederik (2000). "Increased levels of markers of microbial exposure in homes with indoor storage of organic household waste." Appl Environ Microbiol **66**(2): 627-31.