

Exposure to microbial components and allergens in population studies: a comparison of two house dust collection methods applied by participants and fieldworkers

Abstract Dust collection by study participants instead of fieldworkers would be a practical and cost-effective alternative in large-scale population studies estimating exposure to indoor allergens and microbial agents. We aimed to compare dust weights and biological agent levels in house dust samples taken by study participants with nylon socks, with those in samples taken by fieldworkers using the sampling nozzle of the Allergology Laboratory Copenhagen (ALK). In homes of 216 children, parents and fieldworkers collected house dust within the same year. Dust samples were analyzed for levels of allergens, endotoxin, (1 → 3)- β -D-glucans and fungal extracellular polysaccharides (EPS). Socks appeared to yield less dust from mattresses at relatively low dust amounts and more dust at high dust amounts than ALK samples. Correlations between the methods ranged from 0.47–0.64 for microbial agents and 0.64–0.87 for mite and pet allergens. Cat allergen levels were two-fold lower and endotoxin levels three-fold higher in socks than in ALK samples. Levels of allergens and microbial agents in sock samples taken by study participants are moderately to highly correlated to levels in ALK samples taken by fieldworkers. Absolute levels may differ, probably because of differences in the method rather than in the person who performed the sampling.

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Practical Implications

Dust collection by participants is a reliable and practical option for allergen and microbial agent exposure assessment. Absolute levels of biological agents are not (always) comparable between studies using different dust collection methods, even when expressed per gram dust, because of potential differences in particle-size constitution of the collected dust.

Introduction

Epidemiological studies on allergies and asthma typically estimate exposure to allergens by analyzing samples of settled house dust. In this way, relationships between house dust mite allergens and allergy have already been established since the 1960s (Custovic et al., 2003; Huss et al., 2001; Lau et al., 1989, 2000; Tovey, 2004; Voorhorst et al., 1967). During the last decade it has also become more and more common to measure microbial agents, like bacterial endotoxin, in house dust samples as it has been hypothesized that such agents might protect against allergies (Von Mutius et al., 2000). For example, a study in farm and non-farm children showed an inverse relation between endotoxin levels in house dust and atopic sensitization, atopic asthma and hay fever in children, while non-atopic asthma appeared to be more prevalent at higher endotoxin levels (Braun-Fahrlander et al., 2002). In most studies fieldworker-collected dust samples have been analyzed, but in some, including our own PARSIFAL (prevention of allergy-risk factors for sensitization in children related to farming and anthroposophic lifestyle) study (Alfvén et al., 2006; Schram et al., 2005; Schram-Bijkerk et al., 2005), both fieldworkers and study participants collected house dust in partially overlapping populations.

Fieldworkers collected dust by vacuuming with the sampling nozzle of the Allergology Laboratory Copenhagen (ALK, Horshølm, Denmark) loaded with a filter, to assess allergens and microbial agents in house dust of the PARSIFAL population (Schram et al., 2005). Sampling with ALK devices is one of the most commonly used dust sampling methods, which was, for example, also applied in the International Study of Asthma and Allergies in Childhood (ISAAC; Weiland et al., 2004). Participants of the PARSIFAL study collected dust by vacuuming with nylon sample bags (Schram-Bijkerk et al., 2005) ('socks'; Allied Screen Fabrics, Hornsby, Australia), which could, in contrast to ALK devices, be easily mailed to participants, who after sampling returned the socks with dust. This procedure has important logistical advantages for large-scale population studies such as PARSIFAL: the labor costs are much lower than those associated with home visits by fieldworkers, and therefore the size of the population with measured exposure data can be increased considerably. However, little is known about the reliability of such dust sampling by participants: does it lead to similar results as dust collection by fieldworkers? And does the sock itself lead to similar results as ALK nozzles with filters?

The latter question has been addressed previously in a study in New Zealand, which compared allergen and endotoxin levels in parallel mattress and living room dust samples from homes of 37 children, taken on the same day and by the same fieldworker, with either

socks or ALK devices (Wickens et al., 2004). Moderate to high correlations were observed between the levels of endotoxin, cat and mite allergens per gram dust in the two types of samples (Pearson's $r = 0.58\text{--}0.82$).

Another recent study addressed the question whether dust sampling by participants leads to similar results as dust sampling by fieldworkers. Cat and mite (Der p1) allergen levels in dust samples collected by participants were compared with levels in dust samples collected by fieldworkers (Arbes et al., 2005). Strong correlations between the allergen levels were observed, but participants collected lighter samples, which might have been because of the use of different vacuum cleaners or the use of different dust collection devices: the Mitest Dust Collector (participants) and a paper filter method (fieldworkers). In addition, other methods for dust collection by participants have been developed, i.e. dust sampling using electrostatic wipes (Thorne et al., 2005), but this method has, to our knowledge, not been compared with dust sampling by fieldworkers.

In this study, we primarily aimed at comparing the two dust sampling methods applied in the PARSIFAL study: dust sampling with socks by participants and dust sampling with ALK devices by fieldworkers. The comparison involves a wide spectrum of biological agents: mite allergens of *Dermatophagoides pteronyssinus* (Der p1) and *Dermatophagoides farinae* (Der f1), cat (Fel d1) and dog (Can f1) allergens, endotoxin and two mold components: (1 → 3)-β-D-glucans and extracellular polysaccharides (EPS) from *Aspergillus* and *Penicillium* spp. Additionally, we investigated whether differences between the methods could be attributed to the use of different sampling devices by including collection and analysis of extra sock samples, taken by fieldworkers on the same day as the ALK samples.

Methods

Study subjects

The design and population characteristics of the PARSIFAL study have been described previously (Alfvén et al., 2006). In short, children from farmers' families, anthroposophic families ('Steiner children') and respective reference families of five European countries, Austria, Germany, the Netherlands, Sweden and Switzerland, were included (Alfvén et al., 2006). Farm reference and Steiner reference children were children who lived in the neighborhood of farm and Steiner children, but who did not actually live on a farm, or did not attend a Steiner school, respectively (Alfvén et al., 2006). Parents of participating children who gave consent to dust sampling, with a maximum of 1000 per country (the PARSIFAL target population), were sent nylon sampling socks ($n = 4892$) to collect dust with. Nearly 75% ($n = 3625$) of sock sampling sets with dust were returned. The

contents of these house dust samples were analyzed in a case-control selection of nearly 1000 children, to assess the association between exposure to microbial agents and wheeze, as described previously (Schram-Bijkerk et al., 2005). In addition, fieldworkers collected house dust with ALK sampling devices in a randomly selected population, stratified by subgroup (farm, Steiner and reference children) to assess levels of microbial agents in each subgroup (Schram et al., 2005) and to validate the sock method. In short, mattress and living room dust was collected in homes of 478 children: 229 farm children, 122 Steiner children and 60 and 67 of their respective references.

Parent-collected socks from 73 homes, which had been analyzed within PARSIFAL subprojects other than the previously described case-control study (Schram-Bijkerk et al., 2005), were also included in the comparisons with ALK samples. About half of

these additional samples (from 30 homes) were only analyzed for dust weights, endotoxin and EPS levels. Overall, both sock and ALK results were available for 216 homes. In more than 75% of these homes, socks had been collected first, and time between the ALK sampling and sock sampling by parents ranged from -128 to 251 days, with a median of 103 days.

To allow a comparison of sampling devices used by the same person on the same day, fieldworkers collected house dust with socks along with ALK sampling in 114 homes. These homes were selected on the basis of floor covering: only homes with wall-to-wall carpets were included in this additional sampling procedure, because low dust weights, as collected on smooth floors, could make comparisons of side-by-side sampling less reliable. The selection of populations for the various dust sampling procedures has been summarized in a flow-chart (Figure 1).

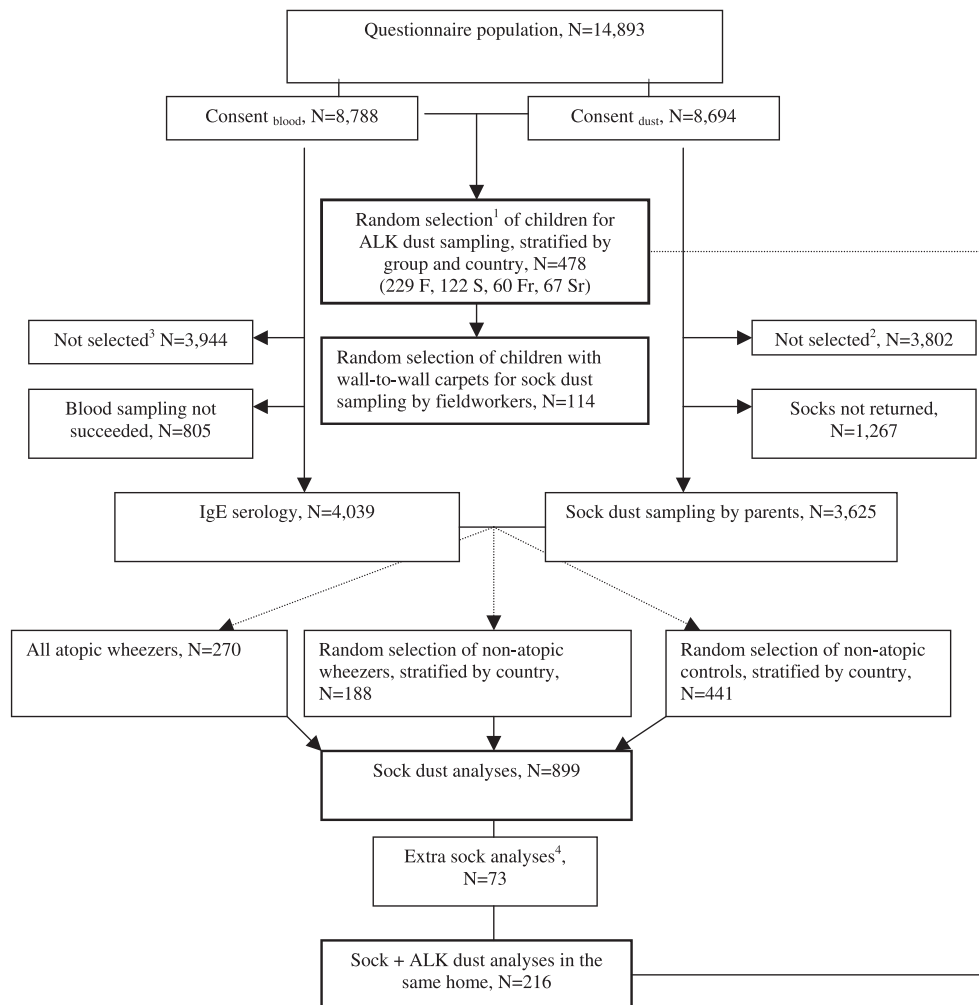


Fig. 1 Schematic overview of the PARSIFAL population, indicating which subpopulations were included in dust sampling (F = Farm, S = Steiner, Fr = Farm reference, Sr = Steiner reference children) ¹Farms without livestock were excluded. AU: only consent to dust sampling required. NL + SE: restrictions to some geographical areas. ²CH and GE made selections, because of their relatively large populations. CH: 1000 children, incl. all with consent to dust and blood sampling; GE: 350 F (consent to dust + blood), 368 S, 150 Fr, 170 Sr. ³Selection of: CH 865 children + 38 extra wheezers; GE: all F, 368 S (incl all wheezers), 150 Fr, 150 Sr (incl all wheezers). (SE = Sweden, CH = Switzerland, NL = the Netherlands, GE = Germany, AU = Austria) ⁴Selected for other PARSIFAL subprojects. Half of these samples (from 30 homes) only analysed for dust weights, endotoxin and EPS.



Fig. 2 ALK sampling nozzle

Dust collection

Fieldworkers collected dust with vacuum cleaners equipped with ALK (Horshølm, Denmark) sampling nozzles (Figure 2), loaded with glass fiber filters, as described previously (Schram et al., 2005) and with nylon sample socks with 25 μm pore size (Allied Screen Fabrics, Hornsby, Australia) (Figure 3). The sample sock was placed into the suction pipe, the upper part of the sock was folded around the outside of the suction pipe and attached with adhesive tape. Samples from mattresses were obtained by vacuuming the whole area of the mattress with undersheets for 2 min twice, with both devices, with the order effect controlled for by alternating the first used device. Duplicate living room samples were obtained by vacuuming two adjacent 1 m^2 sites on the carpet for 2 min each, with different devices. Other types of floor covering were sampled with ALK devices only: smooth floors with ≥ 4 m^2 rug, 1 m^2 of rug, 2 min; smooth floors with no rug or smaller rug(s), 2 m^2 of smooth floor, 4 min. Filters with dust were transferred to pre-weighed tubes and stored frozen at the various centers, and shipped on dry ice to one laboratory (IRAS, Utrecht, the Netherlands) for central analysis.

Parents collected dust with nylon sample socks, applying the same sock sampling procedures as fieldworkers, which were illustrated in detailed photo-instructions (available on request from the corresponding author). They were instructed to vacuum the whole area of the mattress with one sock and 1 m^2 of either carpet, smooth floor or rug in the living room with the other sock, for 2 min each. Socks with sampled dust were returned to the local study centers by regular mail, where they were stored frozen. The samples were shipped on dry ice to Utrecht.

Dust analysis

The tubes with ALK filters plus dust were post-weighed and 5–40 ml extraction fluid was added, 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). The filter was thus extracted as well. The dust in the sock samples was transferred from the sock to a pre-weighed tube and after post-weighing the tube with dust, 5–40 ml extraction fluid was added according to the same weight-fluid scheme as used for ALK samples. Allergens and microbial agents were extracted from the dust in the same way for socks as for ALK samples, according to a three-step procedure, extracting endotoxin first, followed by EPS and allergen extraction and finally extraction of glucans, as described previously (Schram et al., 2005). Endotoxin was measured with the kinetic chromogenic Limulus amoebocyte lysate (LAL) test, EPS with a specific sandwich EIA for EPS of *Aspergillus* and *Penicillium* spp. (Douwes et al., 1999) and glucans with an inhibition EIA (Douwes et al., 1996). Mite allergens of *D. pteronyssinus* (Der p1) and *D. farinae* (Der f1) and the major cat (Fel d1) and dog (Can f1) allergens were measured with sandwich EIAs of Indoor Biotechnologies® as described previously (Van Strien et al., 2002). The average inter-day/inter-assay coefficients of variation, as determined by testing duplicate extract aliquots of 10% of all samples on another day as the first aliquot, were: 30.7% for endotoxin, 13.5% for EPS, 23.4% for glucans, 19.3% for Der p1, 17.6% for Der f1, 18.6% for Fel d1 and 25.9% for Can f1.

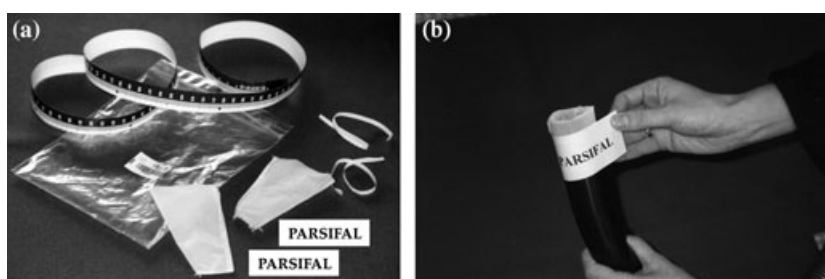


Fig. 3 Sampling set for collection of house dust, including two sample socks, a measuring rod, two stickers and twist ties (a) and attachment of a sock to the vacuum cleaner with a sticker (b)

Data analysis

Results were expressed as concentrations per gram of dust and per square meter. For samples with undetectable amounts of dust (< 0.020 g, $< 5\%$ of all samples), no concentrations per gram of dust were calculated. Samples with non-detectable amounts of dust or biological components were not included in the quantitative comparisons between methods regarding that specific, non-detectable component. Percentages of non-detectable values were low for microbial agents ($< 4\%$) and cat allergens ($< 6\%$), moderate for dog allergens (11–35%) and high for house dust mite allergens (16–24% of mattresses; 43–48% of floor samples). The majority of samples with non-detectable levels of mite allergens were samples from Sweden, which has known low house dust mite allergen levels. Dust collection methods were also evaluated by comparing the sum of Der p1 and Der f1, because this parameter was used in previous analyses in the PARSIFAL population. In quantitative comparisons of the sum of Der p1 and Der f1, all samples with a detectable amount of at least one of the two allergens were included.

Statistical analyses were performed with SAS statistical software (version 8.2, SAS Institute, Cary, NC, USA). Levels of allergens and microbial agents were approximately log-normally distributed after exclusion of samples with non-detectable levels. Pearson correlation coefficients were calculated for ln-transformed values of dust and biological agent levels. To evaluate the agreement between dust sampling methods and to test whether differences depended on the level of the agents, Bland-Altman plots (Bland and Altman, 1999) were produced. In these plots, differences between ln-transformed levels between the methods were plotted against the mean level. Differences in ln-transformed levels were further evaluated by performing *t*-tests for paired observations and they were also plotted against the time interval between dust collection by parents and fieldworkers. We primarily compared dust and biological agent levels in parent-collected socks with levels in ALK samples taken by fieldworkers. Additionally, levels in ALK samples were compared with levels in socks, collected on the same day by fieldworkers.

Results

Dust yields

Parent-collected socks vs. fieldworker-collected ALK samples The amounts of mattress dust were on average 1.5 times lower in parent-collected socks than in ALK samples (0.31 vs. 0.47 g, $P < 0.01$). The difference was in fact underestimated, because 30 of these ALK samples had been taken directly after sock sampling by

the fieldworker; if these 30 samples were excluded, mean ALK mattress dust weights were 0.50 g. For living room floors, mean dust weights were similar in socks collected by parents and ALK samples (0.32 vs. 0.35 g, $P = 0.43$). Pearson correlation coefficients for the amounts of dust in the two types of samples were low to moderate: 0.24 for mattresses and 0.43 for living room floors. Figure 4 shows that for living rooms, the difference between measurements did not depend on the mean dust weight, whereas it appeared that mattress socks showed three to 15 times lower dust weights at low mean dust amounts. In contrast, at high mean mattress dust amounts, sock dust weights were two to three times higher.

Fieldworker-collected sock and ALK samples Amounts of mattress dust in fieldworker-collected socks were 1.7 times lower ($P < 0.01$) than in ALK samples, collected by the same fieldworker on the same day, whereas living room floor dust amounts were not significantly different. The order in which the two mattress samples were taken determined the dust yield: dust amounts in socks were 1.2 times lower when the sock method was

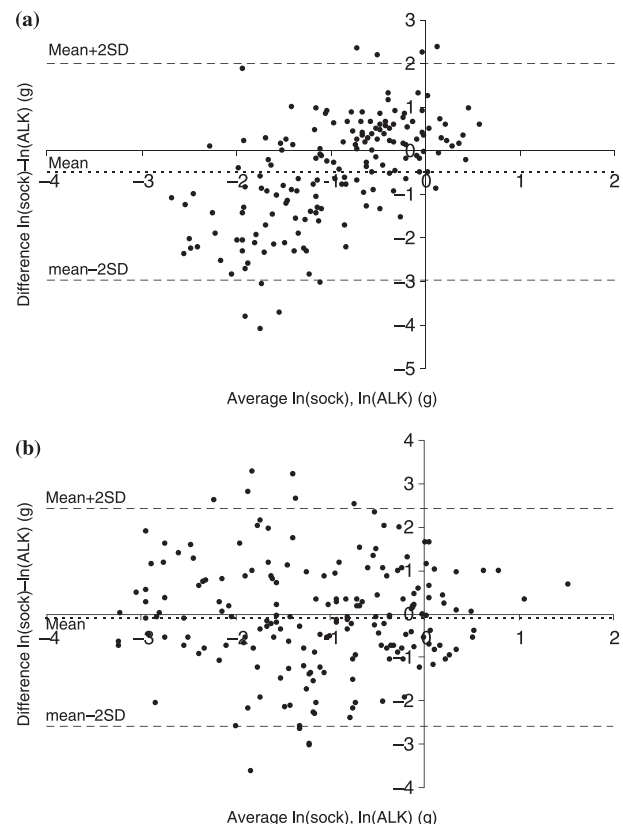


Fig. 4 Bland-Altman plots showing differences between ln-transformed dust weights in parent-collected socks and fieldworker-collected ALK samples^a, for (a) the mattress (b) the living room (s.d. = standard deviation). (a) Thirty mattress ALK samples taken on the same day, shortly after sampling with socks by fieldworkers, were excluded

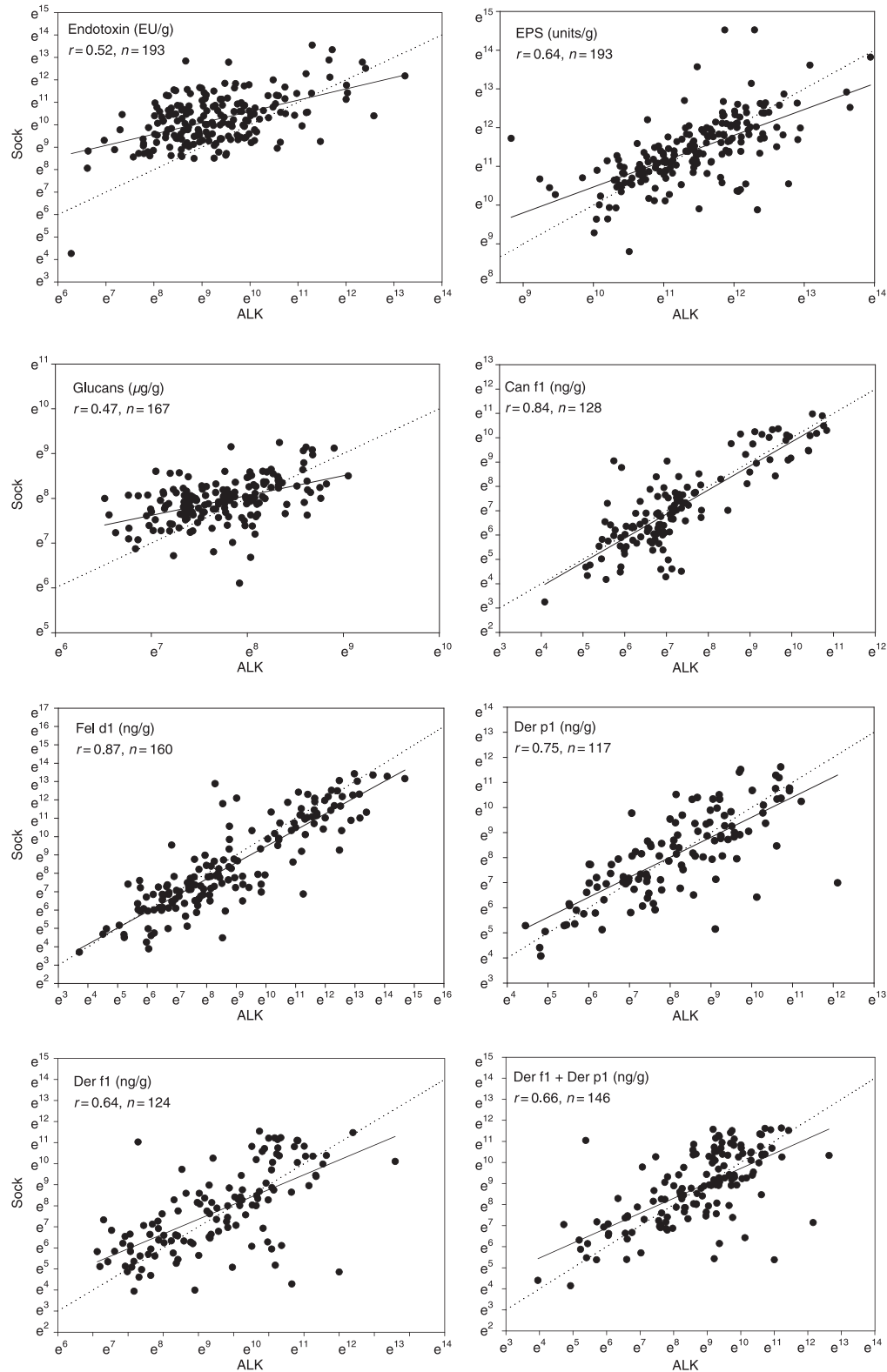


Fig. 5 Correlations between mattress levels of biological agents per gram dust in ALK samples and parent-collected socks, with Pearson correlation coefficients (r), regression line (solid line) and line of unity (dotted line)

applied to the mattress first, but 2.1 times lower when the ALK device was applied first. The Bland-Altman plot for mattress dust weights of fieldworker-collected

socks and ALK samples showed the same trend as Figure 4 for parent-collected socks and ALK samples, with relatively low sock dust weights at low mean dust

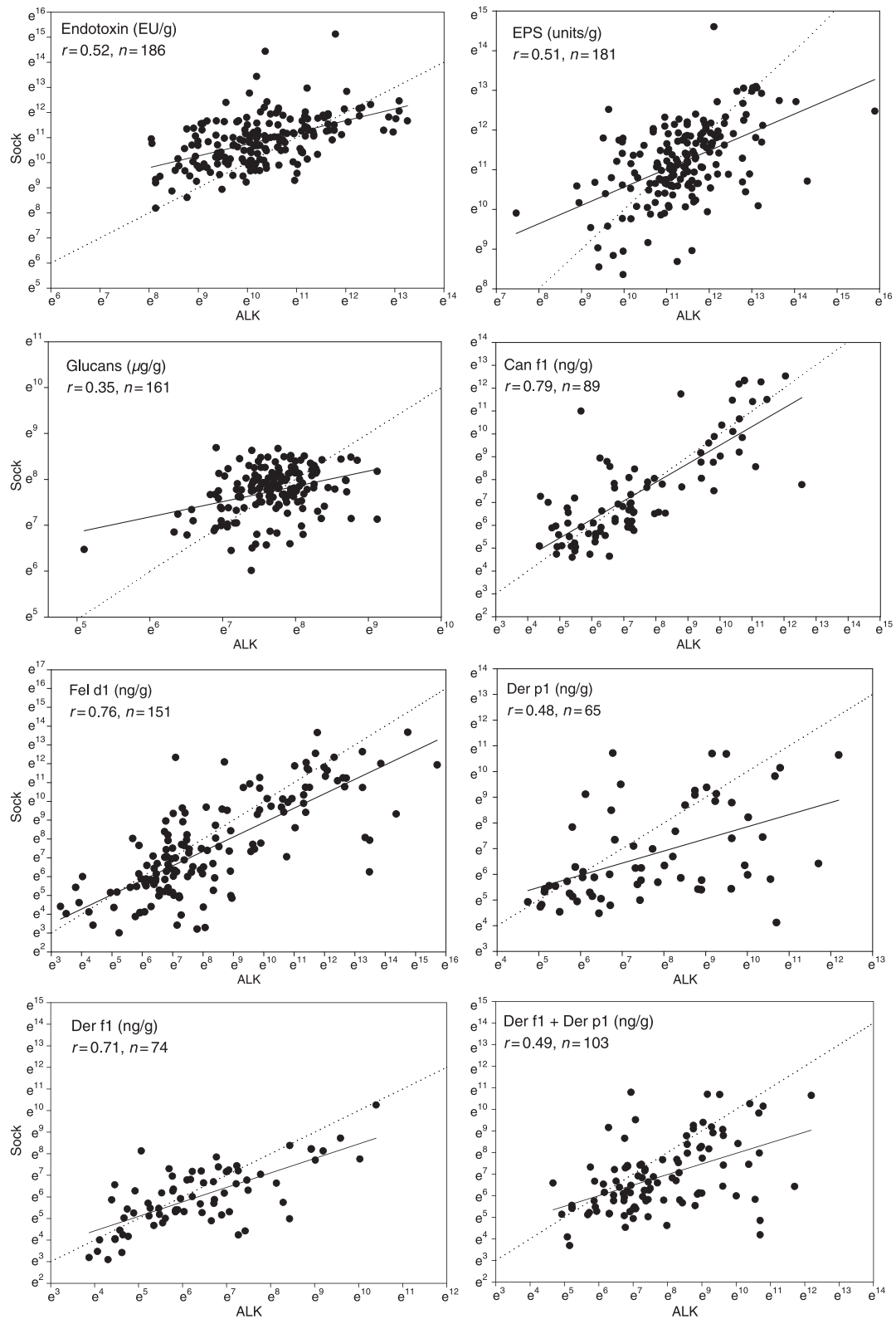


Fig. 6 Correlations between living room floor levels of biological agents per gram dust in ALK samples and parent-collected socks, with Pearson correlation coefficients (r), regression line (solid line) and line of unity (dotted line)

levels (not shown). Pearson correlation coefficients between fieldworker-collected sock and ALK dust levels were, on the other hand, higher than those

between parent-collected socks and ALK samples: r -value was 0.70 for mattress dust and 0.79 for living room floor dust.

Biological agent levels

Parent-collected socks vs. fieldworker-collected ALK samples Figure 5 shows correlations between levels of biological agents per gram of mattress dust measured in ALK samples and parent-collected socks. Levels of endotoxin, EPS and glucans per gram dust were moderately correlated between the sampling methods (Pearsons' r ranging from 0.47 to 0.64). Correlations were high for allergen levels (r 0.64–0.87). Generally, correlations were higher with wider ranges in levels of the specific component; the 10th and 90th percentile values of Fel d1, for example, differed more than 500-fold and Fel d1 showed a high correlation coefficient, whereas for glucans this difference was only 4-fold, and the correlation was moderate. Figure 6 shows the correlations for living room floor samples, which were generally somewhat lower than for mattresses, but with the same overall pattern for the various agents. When allergen and microbial agent levels were expressed per square meter of sampled surface (Table 1), correlation coefficients for floor samples were very similar to those found per gram dust, whereas for mattress samples, the correlation coefficients for levels per square meter were consistently lower, in accordance with the above mentioned lower correlation between dust yields from mattresses, compared with those from living room floors.

Correlations between biological agent levels were similar when levels below the limit of detection, given a value of two-third of the lowest detectable amount, were included in the analyses (not shown). For example, for Can f1, Der p1 and Der f1, which were less often detected than the other components, correlation coefficients for levels per gram mattress dust were 0.69, 0.84 and 0.76 after inclusion of non-detectable samples, respectively. The concordance (percentage detectable or non-detectable on both occasions) for these agents ranged from 66.3% to 82.0%.

Table 1 Pearson correlation between levels of biological agents in ALK samples and parent-collected socks, expressed per square meter sampled mattress and living room area

	Mattress		Living room	
	Ndet ^a	r-value	Ndet ^a	r-value
Endo (EU/m ²)	201	0.43	203	0.55
EPS (units/m ²)	201	0.43	197	0.47
Gluc (μg/m ²)	174	0.34	174	0.51
Can f1 (ng/m ²)	133	0.70	98	0.70
Fel d1 (ng/m ²)	168	0.74	161	0.71
Der f1 (ng/m ²)	127	0.60	78	0.64
Der p1 (ng/m ²)	124	0.67	65	0.52
Der p1 + Der f1 (ng/m ²)	152	0.61	107	0.58

^aNumber of sample pairs with detectable levels of the specific agent in both sock and ALK samples.

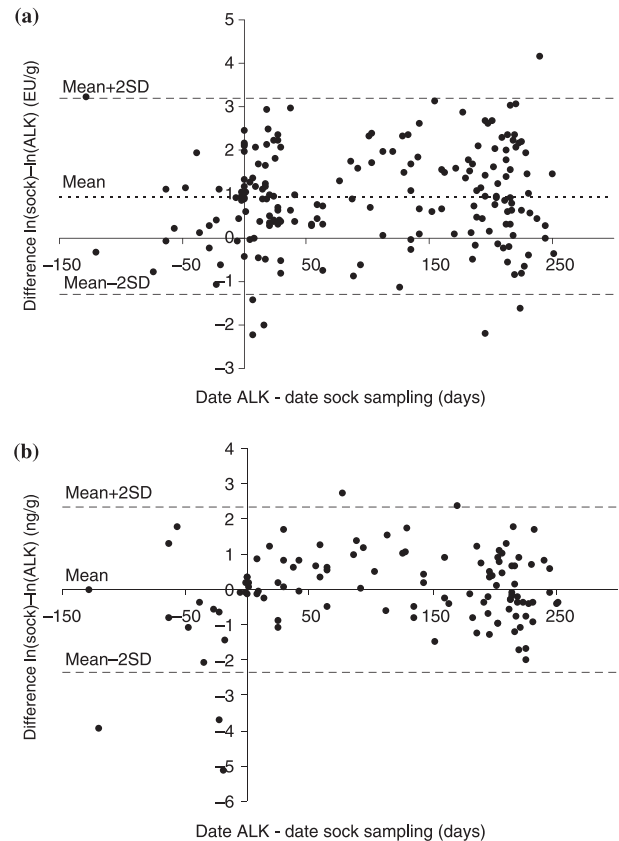


Fig. 7 Bland-Altman plots showing differences between ln-transformed biological agent levels in parent-collected socks and fieldworker-collected ALK samples against the time interval between them, for two examples; (a) endotoxin per gram mattress dust (b) Der p1 per gram mattress dust (s.d. = standard deviation)

Bland-Altman plots in which differences in biological agent levels were plotted against the time interval between sock sampling by parents and ALK sampling by fieldworkers suggested that the time interval played no major role (shown in Figure 7 for endotoxin and Der p1). This was confirmed by restricting correlation calculations to ALK and sock samples taken more than the median number of days apart, which resulted in similar values as those found for the total dataset.

The correlation plots in Figures 5 and 6 show that in general (> 80% of measurements), levels differed up to a factor 7.4 between the methods. Table 3 shows that the mean difference in ln-transformed levels between methods was not significantly ($P < 0.05$) different from 0 for most components in mattress dust. Exceptions were cat allergen levels, which were 1.5–2.0 times lower, both in mattress and living room floor sock samples and endotoxin levels, which were 1.8–2.6 times higher in sock than in ALK samples. Glucan levels were higher in sock mattress samples (Table 3), but not in sock living room samples (not shown), compared with ALK samples. Bland-Altman plots showed that mean differences were not dependent on the mean concentrations (not shown).

Table 2 Pearson correlation between levels of biological agents per gram dust in ALK samples and fieldworker-collected socks

	Mattress		Living room	
	Ndet ^a	r-value	Ndet ^a	r-value
Endo (EU/g)	98	0.66	104	0.57
EPS, EPS (units/g)	99	0.58	106	0.67
Gluc (μ g/g)	99	0.59	105	0.27
Can f1 (ng/g)	74	0.84	76	0.87
Fel d1 (ng/g)	96	0.93	101	0.92
Der f1 (ng/g)	79	0.91	68	0.79
Der p1 (ng/g)	71	0.93	56	0.89
Der p1 + Der f1 (ng/g)	88	0.92	84	0.85

^aNumber of sample pairs with detectable levels of dust and the specific agent in both sock and ALK samples.

Fieldworker-collected sock and ALK samples Correlations between biological agent levels in ALK samples and fieldworker-collected socks (Table 2) were generally higher than correlations between levels in ALK samples and parent-collected socks (Figures 5 and 6). Especially for mite allergens there was a striking difference: Der p1 and Der f1 levels in fieldworker-collected sock and ALK samples showed much higher correlations (r -value 0.79–0.93) than the comparisons of levels of these agents in fieldworker-collected ALK and parent-collected sock samples.

Differences in mean levels of cat allergen and endotoxin were also observed between ALK samples and fieldworker-collected socks (Table 3). Cat allergen levels were 1.7–1.9 times lower ($P < 0.01$) in fieldworker-collected sock mattress and living room samples, and endotoxin levels were 1.2–1.3 times higher ($P < 0.05$ for mattresses and $P < 0.10$ for living rooms) in fieldworker-collected sock samples than in ALK samples. Thus, for endotoxin, differences in levels between fieldworker-collected socks and ALK samples were smaller than those between parent-collected socks and ALK samples, which suggests that, next to sampling device, fieldworker vs. participant dust collection, or the day of dust sampling, influenced

mean levels of endotoxin. No effect of sampling order was seen on levels of biological components per gram mattress dust (not shown).

Discussion

Our data showed that dust collection by participants is a reliable and practical option for allergen and microbial agent exposure assessment, because of high return rates for subject-collected socks (nearly 75%, after one reminder phone-call if necessary) and moderate to high correlations with levels of biological components in fieldworker-collected dust. This paper confirmed previous findings of allergen levels in participant- and fieldworker-collected dust samples (Arbes et al., 2005), whilst adding information of microbial agent levels. We, however, observed differences in absolute levels of some components between the methods, i.e. in dust weights and cat allergen and endotoxin levels per gram dust, which may not be important for large epidemiological studies into relative exposure differences across groups, but which might result in misclassification in other studies. Our data do not provide a direct answer to the question to what extent differences in levels between parent-collected socks and ALK samples could be attributed to either the time interval or the person who performed the sampling (fieldworker vs. parent). However, we included comparisons with socks collected by fieldworkers to investigate the effect of using different devices.

Person who performs the sampling: fieldworkers vs. participants

Although dust yields from socks were in the same order of magnitude as dust amounts from ALK samples, correlations between the two methods were only moderate for dust weights, which is in line with a previous report of participant- and fieldworker-collected dust samples (Arbes et al., 2005). Differences between yields of fieldworker- vs. participant-collected dust samples might be because of the use of different kinds of vacuum

Table 3. Mean biological agent levels per gram mattress dust in parent-collected socks vs. fieldworker-collected ALK samples and in fieldworker-collected socks vs. fieldworker-collected ALK samples

	Parent-collected socks vs. ALK samples			Fieldworker-collected socks vs. ALK samples		
	Ndet ^a	GM (GSD), sock parents	GM (GSD), ALK	Ndet ^a	GM (GSD), sock fieldworker	GM (GSD), ALK
Endo (EU/g)	193	27,689 (3.1)**	10,761 (3.2)	98	14,421 (3.0)*	11,538 (3.3)
EPS, EPS (units/g)	193	89,306 (2.4)	89,324 (2.3)	99	79,358 (2.5)	77,743 (2.2)
Gluc (μ g/g)	167	2830 (1.6)**	2293 (1.7)	99	2834 (1.6)**	2343 (1.7)
Can f1 (ng/g)	128	1290 (5.6)	1492 (4.3)	74	1303 (4.5)	1450 (4.1)
Fel d1 (ng/g)	160	4720 (11.4)**	7107 (10.9)	96	4090 (10.7)**	6755 (9.2)
Der f1 (ng/g)	124	2321 (7.3)	1906 (6.2)	79	2327 (5.4)*	1949 (5.5)
Der p1 (ng/g)	117	3363 (5.6)	3391 (5.1)	71	3639 (6.5)	3761 (6.0)
Der p1 + Der f1 (ng/g)	146	6462 (5.7)	5841 (5.0)	88	7606 (4.8)	7138 (4.7)

^aNumber of sample pairs with detectable levels of dust and the specific agent in both sock and ALK samples.

* $P < 0.05$; ** $P < 0.01$.

cleaners or differences in dust sampling procedures; despite our detailed photo-instructions, it might have been more difficult for parents than for trained fieldworkers to strictly follow the vacuuming instructions (i.e. vacuum 1 m² of the living room floor for exactly 2 min). Our results have not been adjusted for differences in types of vacuum cleaners of study participants, because that information was not collected. However, as dust collection by parents means different vacuum cleaners by definition, it is of more interest to know whether it gives the same results as dust collection by fieldworkers with that variation than without. The low correlations between subject- and fieldworker-collected dust amounts could, however, in our study, also be due the time interval or the device.

Time interval

The time interval between sock sampling by parents and sock/ALK sampling by fieldworkers ranged from -128 to 251 days. One would expect to find larger random differences and thus lower correlations at larger time intervals. We observed higher correlation coefficients for dust yields and mite allergen levels, when dust was collected by fieldworkers on the same day with either ALK or sock devices, which suggests that time interval indeed influenced mean levels, although we could not detect effects in comparing samples taken several days or months apart. Our correlations between levels in parent-collected socks and ALK samples were generally higher than correlations between allergen levels in ALK samples taken 2 years apart (C. Antens, IRAS, Utrecht, unpublished data), which also suggests that time interval between measurements influences correlations.

Different devices

Logically, correlations in this study could not be higher than correlations between levels in duplicate samples, collected on the same day with the same device by the same person. A study comparing such duplicate samples, collected with ALK devices, showed similar correlations as our comparisons of fieldworker-collected socks and ALK samples (Giovannangelo, in press), suggesting that application of sock sampling by fieldworkers leads to similar results as ALK sampling by fieldworkers. This has previously been concluded from a study comparing ALK and sock samples collected by fieldworkers, which assessed endotoxin, mite and cat allergen levels, and which showed similar correlations as our study (Wickens et al., 2004). However, there appeared to be some differences in dust yields and composition of the dust between ALK samples and sock samples, no matter whether socks were taken by fieldworkers or parents. Mattress dust amounts appeared to be lower in socks, particularly at low dust

levels. Sock dust weights cannot have been influenced by vacuum cleaner attachments, because the sock was placed on the suction pipe and sampling was performed directly with the open end of the pipe (Figure 3). ALK sampling of mattresses was easier because the sampling nozzle could be kept at a fixed angle, while with sock sampling the sheets on the mattresses sometimes stuck to the vacuum cleaner pipe and thereby reduced contact with the mattress itself. In addition, dust may have been lost when transferring dust from socks to tubes before extraction, although no differences in dust amounts were observed for living room socks as compared with ALK samples. Interestingly, however, Figure 4 showed that the mattress dust yield did not only depend on collection method, but also on the mean dust weight, with relatively low sock dust weights at low mean dust levels and relatively high sock dust weights at high mean dust levels. Differences in pore sizes (socks 25 µm, filter < 1 µm) might explain this discrepancy. Filters might be more efficient at low dust weights, with large amounts of fine dust, whereas socks might be more efficient in capturing larger particles. The differences were not observed for living room floor dust, suggesting that these differences depend on the constitution of dust.

Cat allergen levels were up to twofold higher in ALK samples than in socks, and endotoxin levels appeared to be up to threefold higher in parent-collected socks than in ALK samples. Cat allergen can be found frequently on particles less than 2.5 µm (De Blay et al., 1991), which might have been captured by glass fiber filters (pores < 1 µm) more easily than by socks (pores 25 µm). Differences in particle size distribution of sock or ALK dust might have influenced endotoxin levels as well. Handling the socks may have contributed to endotoxin levels in the samples, which might have been checked by including field blanks. Although not included in this study, we have noticed in other recent series that endotoxin levels in such control socks are negligible or several orders below that of socks containing at least 50–100 mg dust. Thus, it is hard to imagine that such contamination would explain the threefold difference in endotoxin levels between sock and ALK samples. However, as epidemiological studies usually investigate relative exposure differences between subgroups, e.g. people with and without a particular disease, absolute levels are generally less relevant. Despite differences in absolute levels (i.e. for endotoxin), comparisons of relative microbial exposure differences between groups, i.e. farm children, Steiner children and respective references, showed similar results for ALK samples and socks collected by parents (not shown).

The case-control selection of children in this study might be regarded as limiting the generalizability of our results: the study population consisted of relatively many children with allergies or asthma, which might

have influenced compliance of the parents. On the other hand, it is hard to imagine that a better or lower compliance with procedures would lead to different levels of biological components per gram dust. Therefore, it seems unlikely that it would have biased the relation between levels in samples taken from the same home either by parents using socks or fieldworkers using ALK sampling nozzles.

Conclusions

Dust collection with socks by parents appears to be as valid for allergen and microbial agent exposure assessment as dust collection with ALK sampling devices by fieldworkers. Dust yields, and maybe even dust particle-size distributions may differ between methods, therefore, absolute levels of biological agents are not (always) comparable between studies using different dust collection methods, even when expressed per gram dust.

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Appendix

PARSIFAL (prevention of allergy-risk factors for sensitization in children related to farming and anthroposophic lifestyle) study group: Göran Pershagen, Tobias Alfvén, Johan Alm, Anna Bergström, Lars Engstrand, Helen Flöistrup, Marianne van Hage, Niclas Håkansson, Gunnar Lilja, Fredrik Nyberg, Annika Scheynius, Helena Svensson, Jackie Swartz, Magnus Wickman (Sweden); Charlotte Braun-Fahrlander, Marco Waser, Felix Sennhauser, Roger Lauener, Johannes Wildhaber, Alex Möller (Switzerland); Bert Brunekreef, Dienneke Schram-Bijkerk, Gert Doekes, Mirian Boeve, Jeroen Douwes, Machteld Huber, Mirjam Matze (the Netherlands); Erika von Mutius, Marcus Benz, Jörg Budde, Markus Ege (Germany); Josef Riedler, Waltraud Eder, Ellen Üblagger, Gertraud Weiss, Mynda Schreuer (Austria), Karin Michels (USA).

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