

Variation of biocontaminant levels within and between homes — the AIRALLERG study

MARIËLLA GIOVANNANGELO^a, EMMA NORDLING^{b,c}, ULRIKE GEHRING^{a,d}, MARIEKE OLDENWENING^a, TOM BELLANDER^{b,c}, JOACHIM HEINRICH^d, GERARD HOEK^a AND BERT BRUNEKREEF^{a,e}

^aInstitute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

^bDepartment of Occupational and Environmental Health, Stockholm County Council, Stockholm, Sweden

^cInstitute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

^dGSF, National Research Center for Environment and Health, Neuherberg, Germany

^eJulius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands

Few epidemiological studies report on reliability of exposure measurements even though this significantly influences the results of correlation and regression analysis often used in these studies. Poor reliability of exposure measurement reduces the ability to detect a true association between a certain component and health outcome variables. The aim of this study was to determine the ratio of the within-home and between-home components of variance of a number of biocontaminants measured in house dust in the framework of an international study conducted in the Netherlands, Germany and Sweden (the AIRALLERG study). To this end, duplicate dust samples were collected from children's beds and from living room floors in over 100 homes. Samples were taken at the same point in time. Variables considered were the dust mass collected in mg/m² and the concentrations of the house dust mite allergens *Der p 1* and *Der f 1*, cat allergen *Fel d 1*, endotoxin, (1→3)- β -D-glucan and extracellular polysaccharides, all per gram of dust and per square meter of sampling surface. An analysis of variance showed that the within-home variance was small compared to the between-home variance for most variables (mostly less than half) with the exception of glucan on mattresses, when expressed in μ g/g. Investigation of variation over time is needed for a more complete assessment of the use of these variables in epidemiological analyses of exposure–response relationships.

Journal of Exposure Science and Environmental Epidemiology (2007) 17, 134–140. doi:10.1038/sj.jes.7500482; published online 5 April 2006

Keywords: measurement error, exposure assessment, allergens, mould, endotoxin.

Introduction

Many epidemiological studies have related levels of biocontaminants in house dust to allergy and asthma outcomes (Braun-Fahrlander et al., 2002; Bottcher et al., 2003; Takaro et al., 2004). Often these studies made only a brief comment on the reliability of the techniques and assays used to assess exposure to these contaminants. For epidemiological purposes, what matters is whether the true between-person variation is sufficiently captured by the exposure assessment method, or whether this variation is obscured by within-person variability due to errors in sampling and analyses. The error of the analytic method is often small compared to the variability of the exposure measurement in time and space. In correlation and regression analysis commonly used in epidemiological studies, the ratio of the within- (or error)

and between-subject components of variance determines the extent to which correlation and regression coefficients may be biased. Having a large error variance reduces the ability to detect a significant association between exposure and disease as reviewed by Armstrong (1998).

The aim of this study was to determine the ratio of the within-home and between-home components of variance of a number of biocontaminants measured at one point in time in house dust in the framework of an international study conducted in the Netherlands, Germany and Sweden (the AIRALLERG study). We performed an analysis of variance on a large number of duplicate house dust samples. Variables considered were dust mass collected per square meter of sampling surface, and the concentration of the house dust mite allergens *Der p 1* and *Der f 1*, cat allergen *Fel d 1*, endotoxin, (1→3)- β -D-glucan and extracellular polysaccharides, all per (milli)gram of dust and per square meter.

1. Address all correspondence to: Dr. B. Brunekreef, Institute for Risk Assessment Sciences, Universiteit Utrecht, PO Box 80178, 3508 TD, Utrecht, The Netherlands. Tel.: +31 30 2539490. Fax: +31 30 2539499. E-mail: b.brunekreef@iras.uu.nl

Received 29 December 2005; revised 27 February 2006; accepted 27 February 2006; published online 5 April 2006

Methods

The AIRALLERG study was conducted in the framework of four ongoing birth cohort studies of similar design: PIAMA

in the Netherlands (Brunekreef et al., 2002), BAMSE in Sweden (Wickman et al., 2002) and GINI (von Berg et al., 2003) and LISA (Heinrich et al., 2002) in Germany. The AIRALLERG study was designed to obtain environmental samples, including dust samples from mattresses and living room floors, in the homes of 180 sensitized and 180 non-sensitized children in each country. In a subsample of 114 homes, duplicate samples were taken from mattress and/or floor to investigate the reliability of the sampling and analysis methods.

Mattress duplicates were taken from 61 homes and floor duplicates from 58 homes; duplicate dust samples were obtained.

Dust sampling

The dust sampling on living room floor and the child's mattress was performed during mid-January to mid-April (the cool season) of 2002 and mid-October to mid-May of 2002 to 2003 using previously developed procedures (Schram et al., 2005). In short, dust samples were collected with vacuum cleaners equipped with ALK sampling nozzles (Allergologisk Laboratorium, København, Horsholm, Denmark) containing pre-weighed glass fiber filters (70 mm glass fiber filter; Schleicher & Schuell; ref. no. 370104). Duplicate samples from the living room floor were taken from two connecting surfaces of either 1 m² for 2 min of wall-to-wall carpets or rugs of at least 4 m², or from 2 m² of smooth floor (when no carpets or rugs were present) at a rate of 2 min/m². Duplicate samples from the child's mattress were taken by vacuuming half of the top surface of the mattress for 2 min for each duplicate. The surface area of the mattress was also measured. After sampling, the filters with the sampled dust were carefully removed from the ALK filter holder. Greiner 50 ml tubes with the filter and dust were stored at -20°C until post-sampling weighing and extraction.

Extractions of house dust samples

As we were interested in analyzing the dust samples for several different components, we used a sequential extraction method (Douwes et al., 1995). In short, the hydrophilic endotoxin and EPS were extracted using pyrogen free water containing 0.05% tween. After the extraction procedure, 10% of the extraction fluid was removed and stored for analysis. That amount was replenished with 10 times phosphate-buffered saline (PBS) for the extraction of proteins like allergens. After the extraction procedure 10% was removed and stored for analysis. That amount was replenished again with 1 time PBS and then heated to 120°C in order to extract sugar macromolecules like (1→3)-β-D-glucan. All samples were stored at -20°C until analysis.

Assays

The biocontaminants were analyzed using established techniques. Endotoxin, (1→3)-β-D-glucan and EPS in

house dust samples were analyzed using techniques reported by Schram et al. (2005). Bacterial endotoxin was measured with the kinetic Limulus Amebocyte Lysate test (Cambrex Bio Whittaker, kit no. 2L1040, Lysate lot no. K0765). A control standard line of LPS (Cambrex Bio Whittaker LPS standard lot no. 2L0090) and one control LPS (Sigma LPS; E-coli 055:B05, lot no. 316523/1895) were used. Samples were measured in a 1 in 500 dilution. The concentration of endotoxin in sample was expressed as endotoxin units (EU) per gram of dust or EU per square meter. The detection limit of the assay in this study was 0.05 EU/ml. The inhibition Enzyme Immuno Assays (EIA) for mould (1→3)-β-D-glucan (Douwes et al., 1996) and the sandwich EIA for extracellular polysaccharides (Douwes et al., 1999) were developed at IRAS. In short, glucans are measured when diluted purified rabbit anti-Laminarin antibodies with known titer are incubated in Laminarin-coated microwells in the presence of (diluted) test samples or buffer, after which the binding of anti-Laminarin antibodies to the coating is quantified by incubation of peroxidase-labeled anti-IgG antibody (SwzR-Po DAKO P0339) and then developed with a substrate. The detection limit of the assay in this study was 3.3 µg/ml. EPS from two species of mould that predominate in many indoor environments in western Europe (*Penicillium* and *Aspergillus*) was measured by using EPS-specific antibodies as coating and conjugate in a sandwich EIA. The detection limit of the assay in this study was 0.8 EPSU/ml. The procedure for analyzing allergens in house dust samples has been reported before (van Strien et al., 2002). Sandwich EIA that are commercially available were used to measure the two major house dust mite allergens and the major cat allergen (Indoor Biotechnologies; *Der p 1* kit 5H8/4C1 batch 2510, *Der f I* kit 6A8/4C1 batch 2501, *Fel d 1* kit 6F9/3E4 batch 2622). The detection limits of the assays in this study were 4.7 ng/ml for *Der p1*, 3.0 ng/ml for *Der f1* and 1.1 ng/ml for *Fel d1*.

Statistical analysis

The concentrations of the biocontaminants had a right-skewed distribution and therefore were log transformed before analysis of variance with SAS PROC VARCOMP. Dust mass per square meter, and biocontaminants per gram and per square meter were analyzed. Separate calculations were performed by sample location, either mattress or living room floor. Non-detectable samples were given a value of 2/3 of the smallest detectable value. The analyses were conducted for the full data set, and then for the data set limited to the duplicate samples that were both found to be above the detection limit for that particular biocontaminant as a sensitivity analysis. As samples were collected in three different countries, all analyses were adjusted for country. Although samples were collected only in the heating season, we performed an additional adjustment for season, defined as

fall (October–December), winter (January–March) and spring (April–May). As these adjustments for country lead to a somewhat attenuated variation between homes, the net effect is that the ratios of within-home to between-home variance are somewhat increased.

All results were expressed as variance ratios, that is, the ratio of within-home to between-home variance.

Results

Duplicate samples were taken from mattresses in 61 homes (17 in Sweden, 23 in Germany and 21 in The Netherlands)

and from living room floors in 58 homes (18 in Sweden, 22 in Germany and 18 in The Netherlands). Table 1 gives a description of the levels that were found and, Figure 1 provides Box plots of the distributions separately for samples collected from child mattresses and living room floors. Most Swedish samples did not contain detectable mite allergen levels. In Germany and The Netherlands, about 40% of floor samples did not contain detectable mite allergen levels. The large majority of samples did contain detectable levels of endotoxin, glucan, EPS and cat allergen. In Sweden, all floor samples were collected from rugs, which explains the higher dust mass levels compared to the other two countries, as well as the smaller geometric standard deviation.

Table 1. Number of samples below the detection limit, geometric mean and geometric standard deviation of the concentrations of the different components per gram of dust or per square meter

Component	Country	/	Mattress				Floor			
			<i>N</i>	<i>n</i> (%) <LOD	GM	GSD	<i>N</i>	<i>n</i> (%) <LOD	GM	GSD
Dust (mg)	Ge	m ²	46	1 (2)	290	2.8	42	0 (0)	194	4.1
	NL	m ²	42	0 (0)	370	2.1	36	0 (0)	151	5.5
	Sw	m ²	34	0 (0)	249	1.8	36	0 (0)	568	1.5
Endotoxin (EU)	Ge	g	46	2 (4)	9820	2.9	44	1 (2)	18,934	3.4
		m ²	46	2 (4)	3061	4.0	42	1 (2)	3539	5.7
	NL	g	42	0 (0)	11,635	2.3	36	0 (0)	18,152	2.1
		m ²	42	0 (0)	4301	2.7	36	0 (0)	2749	8.4
	Sw	g	34	0 (0)	6918	1.8	36	0 (0)	12,197	2.1
		m ²	34	0 (0)	1725	2.5	36	0 (0)	6925	2.4
Glucan (µg)	Ge	g	46	0 (0)	1839	1.7	44	0 (0)	2420	1.5
		m ²	46	0 (0)	554	2.7	42	0 (0)	472	4.9
	NL	g	42	0 (0)	1416	1.7	36	0 (0)	2255	1.7
		m ²	42	0 (0)	523	2.5	36	0 (0)	342	7.6
	Sw	g	34	0 (0)	2337	1.6	36	0 (0)	2812	1.7
		m ²	34	0 (0)	583	2.0	36	0 (0)	1596	2.2
EPS (EPSU)	Sw	g	34	2 (6)	12,597	4.3	36	0 (0)	60,860	2.2
		m ²	34	2 (6)	3050	6.0	36	0 (0)	34,554	2.7
	Ge	g	46	2 (4)	27,073	4.1	44	5 (11)	25,841	8.8
		m ²	46	2 (4)	8283	7.2	42	4 (10)	7354	14.1
	NL	g	42	0 (0)	26,561	2.7	34	8 (24)	9651	16.7
		m ²	42	0 (0)	9819	4.0	34	8 (24)	2295	42.8
<i>Fel d 1</i> (ng)	Ge	g	46	3 (7)	695	8.3	44	4 (9)	726	25.3
		m ²	46	3 (7)	190	11.4	42	3 (7)	162	36.1
	NL	g	42	0 (0)	1886	9.7	36	3 (8)	2271	25.1
		m ²	42	0 (0)	697	11.5	36	3 (8)	371	54.5
	Sw	g	32	1 (3)	3590	18.6	34	0 (0)	701	3.0
		m ²	32	1 (3)	894	21.6	34	0 (0)	386	3.3
<i>Der p 1</i> (ng)	Ge	g	46	21 (46)	155	11.6	44	27 (61)	84	8.5
		m ²	46	21 (46)	49	14.0	44	27 (61)	20	7.1
	NL	g	42	3 (7)	956	4.3	36	19 (53)	113	9.3
		m ²	42	3 (7)	379	5.9	36	19 (53)	35	12.7
	Sw	g	34	27 (79)	31	3.2	34	31 (91)	21	1.8
		m ²	34	27 (79)	9	3.0	34	31 (91)	7	2.3
<i>Der f 1</i> (ng)	Ge	g	46	11 (24)	460	11.8	44	17 (39)	182	12.5
		m ²	46	11 (24)	148	14.7	44	17 (39)	42	13.1
	NL	g	42	5 (12)	1106	10.5	36	14 (39)	160	10.2
		m ²	42	5 (12)	380	13.7	36	14 (39)	53	13.0
	Sw	g	34	34 (100)	15	1.0	34	28 (82)	24	3.2
		m ²	34	34 (100)	3	1.0	34	28 (82)	6	4.3

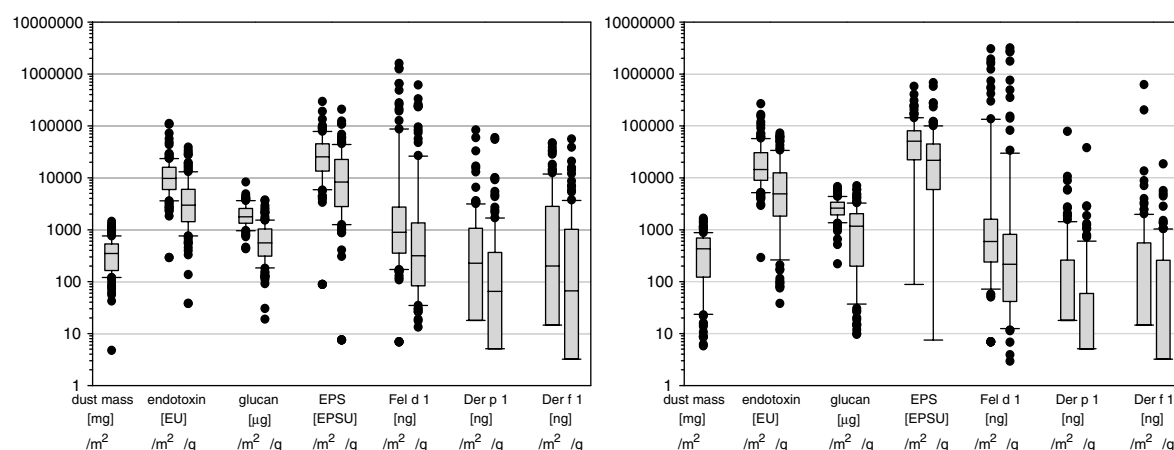


Figure 1. Distribution of biocontaminant levels in mattress dust (left) and living room floor dust samples (right).

Table 2. Results of the analysis of variance

Variable	Location	Number of observations	Number of homes	Within-home variance	Between-home variance	λ
Dust mass (mg/m ²)	Mattress	122	61	0.18	0.48	0.38
	Floor	114	57	0.08	1.47	0.06
Endotoxin (EU/g)	Mattress	122	61	0.16	0.61	0.27
	Floor	116	58	0.32	0.60	0.53
Endotoxin (EU/m ²)	Mattress	122	61	0.24	1.02	0.23
	Floor	114	57	0.21	2.30	0.09
β 1 $\rightarrow$ 3 Glucan (μ g/g)	Mattress	122	61	0.14	0.08	1.64
	Floor	116	58	0.20	0.04	4.69
β 1 $\rightarrow$ 3 Glucan (μ g/m ²)	Mattress	122	61	0.19	0.61	0.32
	Floor	114	57	0.33	1.91	0.17
EPS (EPSU/g)	Mattress	122	61	0.47	1.25	0.38
	Floor	114	57	0.82	3.22	0.25
EPS (EPSU/m ²)	Mattress	122	61	0.68	2.30	0.30
	Floor	112	56	0.44	6.37	0.07
<i>Fel d 1</i> (ng/g)	Mattress	120	60	1.69	4.06	0.42
	Floor	114	57	1.88	5.96	0.32
<i>Fel d 1</i> (ng/m ²)	Mattress	120	60	2.20	4.78	0.46
	Floor	112	56	1.13	9.34	0.12
<i>Der p 1</i> (ng/g)	Mattress	122	61	0.61	2.86	0.21
	Floor	114	57	1.35	2.09	0.64
<i>Der p 1</i> (ng/m ²)	Mattress	122	61	0.56	3.59	0.15
	Floor	114	57	0.80	3.01	0.26
<i>Der f 1</i> (ng/g)	Mattress	122	61	0.85	3.35	0.25
	Floor	114	57	2.23	2.40	0.93
<i>Der f 1</i> (ng/m ²)	Mattress	122	61	0.96	4.24	0.23
	Floor	114	57	1.95	3.41	0.57

λ = ratio of within- and between-home variances, adjusted for country and season. Based on natural-log-transformed data. All duplicates.

The variance ratios for dust mass were low (0.38 for mattresses, 0.06 for floors) (see Table 2) even though the coefficient of variation of the measurement itself was above 20% (data not shown).

Only two variance ratios were above 1, indicating a larger within-home variance than between-home variance, and this was for glucan, when expressed in μ g/g. Most of the other variance ratios were below 0.5, indicating that within-home

variance was less than half of the between-home variance. The results were slightly more favorable for biocontaminant levels expressed per square meter than levels expressed per gram of dust.

As a sensitivity analysis, we also calculated variance ratios using only those duplicate samples that were both above the detection limit (Table 3). The results indicate that for *Der f 1* allergen on living room floors, variance

Table 3. Results of the analysis of variance

Variable	Location	Number of observations	Number of homes	Within-home variance	Between-home variance	λ
Dust mass (mg/m ²)	Mattress	120	60	0.10	0.46	0.21
	Floor	114	57	0.08	1.47	0.06
Endotoxin (EU/g)	Mattress	120	60	0.16	0.39	0.42
	Floor	114	57	0.22	0.55	0.41
Endotoxin (EU/m ²)	Mattress	120	60	0.24	0.74	0.33
	Floor	112	56	0.21	2.19	0.10
β 1 $\rightarrow$ 3 Glucan (μ g/g)	Mattress	122	61	0.14	0.08	1.64
	Floor	116	58	0.20	0.04	4.69
β 1 $\rightarrow$ 3 Glucan (μ g/m ²)	Mattress	122	61	0.19	0.61	0.32
	Floor	114	57	0.33	1.91	0.17
EPS (EPSU/g)	Mattress	116	58	0.14	0.57	0.25
	Floor	98	49	0.14	0.57	0.25
EPS (EPSU/m ²)	Mattress	116	58	0.28	1.29	0.22
	Floor	98	49	0.17	2.04	0.08
<i>Fel d 1</i> (ng/g)	Mattress	112	56	0.34	4.43	0.08
	Floor	100	50	0.49	6.17	0.08
<i>Fel d 1</i> (ng/m ²)	Mattress	112	56	0.33	5.02	0.07
	Floor	100	50	0.58	7.89	0.07
<i>Der p 1</i> (ng/g)	Mattress	62	31	0.22	1.84	0.12
	Floor	26	13	0.37	1.76	0.21
<i>Der p 1</i> (ng/m ²)	Mattress	62	31	0.19	2.74	0.07
	Floor	26	13	0.46	2.34	0.20
<i>Der f 1</i> (ng/g)	Mattress	64	32	0.23	2.30	0.10
	Floor	44	22	1.50	0.91	1.65
<i>Der f 1</i> (ng/m ²)	Mattress	64	32	0.21	3.15	0.07
	Floor	44	22	1.45	1.23	1.18

λ = ratio of within- and between-home variances, adjusted for country and season. Based on natural-log-transformed data. Only homes with two samples > LOD.

ratios increased. For all other variables, there was little change.

Variance ratios were smaller when floor samples were taken from carpeted than from smooth floors. This was especially evident for the cat and mite allergens, and the differences were less for endotoxin, glucan and EPS.

Discussion

We analyzed the within- and between-home components of variance of biocontaminant levels in a large set of duplicate house dust samples taken in three different countries. In general, the within-home variance was small compared to the between-home variance, despite some large coefficients of variation of the laboratory analyses. For epidemiological analyses of exposure-response relationships, the ratio of within- and between-home components of variance is the most important issue to consider. In other large-scale studies of allergens, endotoxin, glucans and EPS in house dust, we also found that the variation between homes was usually much larger than the coefficient of variation of the laboratory procedures (Van Strien et al., 2002; Schram et al., 2005).

Other researchers have also looked into the variability in space. Loan et al. collected dust from 16 living room floors

that were divided into sections of 1 m² and analyzed each section for dust weight and concentrations of *Der p 1* and *Fel d 1*. The authors found that the assumption that a sample from a central area of the living room floor was representative of the whole room was correct for *Der p* and *Fel d 1* (Loan et al., 2003). Simpson et al. however found considerably larger variability for the living room and bedroom floors for *Der p 1* but this variability was less in the immediate area around soft furnishings and beds than the rest of the room (Simpson et al., 1998). Formal analyses of components of variance were not presented by these authors. As we sampled adjacent areas of the living room floor, it is possible that the variance ratios we found were lower than we would have found when sampling from more distant locations. We chose to sample the best accessible locations, which are also the locations children are most likely to spend time on. For mattresses, we sampled two halves of the surface. Usually, the whole surface is sampled in studies of this kind, so that the variance ratios we found for mattresses are likely somewhat higher than for samples taken from the complete surface.

In this study, we were only able to analyze the variance of duplicate samples taken at the same time in the home. Other researchers have also looked into variations over time and performed repeated sampling over 1 year (Chew et al., 1999;

Heinrich et al., 2003) or over 6 years (Topp et al., 2003). Chew et al. (1999) found much smaller within-home standard deviations for *Der p 1*, *Der f 1* and *Fel d 1* than between-home standard deviations. Heinrich et al. (2003) reported correlations between repeated floor dust samples of 0.75, 0.65 and 0.59 for cat allergen, mite allergen and endotoxin, respectively, when expressed per gram of dust. Correlations were 0.74, 0.65 and 0.67, respectively, when contaminants were expressed per square meter. Over a 6-year period, these investigators found that within-home variance was still no larger than between-home variance (Topp et al., 2003) for mite and cat allergen as well as endotoxin. Over a period of 3–24 months, Hirsch et al. (1998) found that the ratio of within- to between- home variance for *Der p 1* and *Der f 1* varied from 0.15 to 0.55 for floors and mattress samples, either expressed per gram of dust or per square meter. This is comparable to the findings for our concurrent duplicate samples.

Similar findings have been reported from a study conducted in the US that investigated endotoxin in mattress dust over a period of 13 months (Park et al., 2000). Very little work has been carried out with EPS in general and only one study looked into the within- and between-home variance of EPS. Chew et al. (2001) found lower within-home variance than between-home variance for EPS for samples taken every 6 weeks for a period of 9 months.

Taken together, the results of our study and those reported by others suggest that the within-home variance of biocontaminants assayed in a single house dust sample is usually, and often considerably, less than the between-home variance.

The results for the glucans were poor when expressed per gram of dust both on the child's mattress and living room floor. The within-home variance was close to or higher than the between-home variance. Chew et al. (Chew et al., 2001) found the same near-similar within-home and between-home variances for the glucan in their bedroom floor samples. The glucan concentrations in Chew's study were expressed per gram of dust. In this study, it was seen that when expressed per square meter of mattress or floor the correlation improved dramatically. Apparently, most of the between-home variance in glucan levels per square meter is explained by the between-home variance of the dust mass sampled per square meter.

There is a substantial body of work concerned with the effects of measurement error of a variety of environmental and occupational exposures in studies, epidemiological or otherwise including many ways of dealing with measurement error in several kinds of statistical analysis, analyses of study power and sample size and the consequences of ignoring measurement error. This has been comprehensively reviewed by Armstrong et al. (1992) and White et al. (1994). For instance, a variance ratio of 1 would lead to a halving of the regression coefficient in a regression analysis.

Ideally, exposure assessment strategies should be designed to increase feasibility and decrease participant burden and overall cost. A decision must be made on how far the investments into the precision need to go to optimize study power. For this, a validity or reliability substudy needs to be performed to see when the proportional increase in total study cost per subject exceeds the proportional gain in reliability (Armstrong, 1995, 1996).

In conclusion, our study showed that across a wide range of circumstances, single measurements of a series of biocontaminants in mattress and floor dust are sufficiently precise to represent biocontaminant levels in these respective dust reservoirs at one point in time. Further work is needed to assess variation of biocontaminant levels over time in homes.

Acknowledgements

Primary funding for the AIRALLERG study was provided by the European Union in the framework program Quality of Life and Management of Living Resources under Key action 4: Environment and Health, Project Reference: QLK4-2000-00073. The following people are greatly thanked for their tireless efforts in the laboratory: Mirian Boeve, Gert Buurman, Kathelijne van Rijswijk, Jack Spithoven, Griet Terpstra and Siegfried de Wind. The local fieldwork teams made it all possible.

References

- Armstrong B. Study design for exposure assessment in epidemiological studies. *Sci Total Environ* 1995; 168: 187–194.
- Armstrong B.G. Effects of measurement error on epidemiological studies of environmental and occupational exposures. *Occup Environ Med* 1998; 55: 651–656.
- Armstrong B.G. Optimizing power in allocating resources to exposure assessment in an epidemiologic study. *Am J Epidemiol* 1996; 144: 192–197.
- Armstrong B.K., White E., and Saracci R. *Chapter 3: Exposure Measurement Error and Its Effects. Principles of Exposure Assessment in Epidemiology.* Oxford University Press, Oxford, 1992, pp. 49–77.
- Bottcher M.F., Bjorksten B., Gustafson S., Voor T., and Jenmalm M.C. Endotoxin levels in Estonian and Swedish house dust and atopy in infancy. *Clin Exp Allergy* 2003; 33: 295–300.
- Braun-Fahrlander C., Riedler J., Herz U., Eder W., Waser M., and Grize L., et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347: 869–877.
- Brunekreef B., Smit J., de Jongste J.C., Neijens H.J., Gerritsen J., and Postma D.S., et al. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol* 2002; 13: 55–60.
- Chew G.L., Douwes J., Doekes G., Higgins K.M., van Strien R., and Spithoven J., et al. Fungal extracellular polysaccharides, beta (1-3)-glucans and culturable fungi in repeated sampling of house dust. *Indoor Air* 2001; 11: 171–178.
- Chew G.L., Higgins K.M., Gold D.R., Mulenberg M.L., and Burge H.A. Monthly measurements of indoor allergens and the influence of housing type in a northeastern US city. *Allergy* 1999; 54: 1058–1066.
- Douwes J., Doekes G., Montijn R., Heederik D.J.J., and Brunekreef B. Measurement of beta(1→3)-glucans in occupational and home environment with an inhibition enzyme immunoassay. *Appl Environ Microbiol* 1996; 62: 3176–3182.

- Douwes J., van der Sluis B., Doekes G., van Leusden F., Wijnands L., and van Strien R., et al. 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* 1999; 103: 494–500.
- Douwes J., Versloot P., Hollander A., Heederik D.J.J., and Doekes G. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995; 61: 1763–1769.
- Heinrich J., Bolte G., Holscher B., Douwes J., Lehmann I., and Fahlbusch B., et al. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur Respir J* 2002; 20: 617–623.
- Heinrich J., Holscher B., Douwes J., Richter K., Koch A., and Bischof W., et al. Reproducibility of allergen, endotoxin and fungi measurement in the indoor environment. *J Expos Anal Environ Epidemiol* 2003; 13: 152–160.
- Hirsch T., Kuhlisch E., Soldan W., and Leupold W. Variability of house dust mite allergen exposure in dwellings. *Environ Health Perspect* 1998; 106: 659–664.
- Loan R., Siebers R., Fitzharris P., and Crane J. House dust-mite allergen and cat allergen variability within carpeted living room floors in domestic dwellings. *Indoor Air* 2003; 13: 232–236.
- Park J.H., Spiegelman D.L., Burge H.A., Gold D.R., Chew G.L., and Milton D.K. Longitudinal study of dust and airborne endotoxin in the home. *Environ Health Perspect* 2000; 108: 1023–1028.
- Schram D., Doekes G., Boeve M., Douwes J., Riedler J., and Ublagger E., et al. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children — the PARSIFAL study. *Allergy* 2005; 60: 611–618.
- Simpson A., Hassall R., Custovic A., and Woodcock A. Variability of house-dust-mite allergen levels within carpets. *Allergy* 1998; 53: 602–607.
- Takaro T.K., Krieger J.W., and Song L. Effect of environmental interventions to reduce exposure to asthma triggers in homes of low-income children in Seattle. *J Expos Anal Environ Epidemiol* 2004; 14: S133–S143.
- Topp R., Wimmer K., Fahlbusch B., Bischof W., Richter K., and Wichmann H.E., et al. Repeated measurements of allergens and endotoxin in settled house dust over a time period of 6 years. *Clin Exp Allergy* 2003; 33: 1659–1666.
- van Strien R., Koopman L., Kerkhof M., Spithoven J., de Jongste J.C., and Gerritsen J., et al. Mite and pet allergen levels in homes of children born to allergic and non-allergic parents: the PIAMA study. *Environ Health Perspect* 2002; 110: A693–A698.
- von Berg A., Koletzko S., Grubl A., Filipiak-Pittroff B., Wichmann H.E., and Bauer C.-P., et al. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol* 2003; 111: 533–540.
- White E., Kushi L.H., and Pepe M.S. The effect of exposure variance and exposure measurement error on study sample size: implications for the design of epidemiologic studies. *J Clin Epidemiol* 1994; 47: 873–880.
- Wickman M., Kull I., Pershagen G., and Nordvall S.L. The BAMSE project: presentation of a prospective longitudinal birth cohort study. *Pediatr Allergy Immunol* 2002; 13: 11–13.