

Overview of Personal Occupational Exposure Levels to Inhalable Dust, Endotoxin, $\beta(1\rightarrow3)$ -Glucan and Fungal Extracellular Polysaccharides in the Waste Management Chain

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Introduction: In the past decade, we studied occupational bioaerosol exposures in various sites of the waste management chain. In this paper we present an overview of exposure levels of inhalable dust, endotoxin, $\beta(1\rightarrow3)$ -glucan (known or probable inducers of airways inflammation), and extracellular polysaccharide antigens of *Aspergillus* and *Penicillium* species (EPS-Pen/Asp; a common and probably more specific marker of fungal exposure). **Methods:** Over 450 personal bioaerosol samples were taken. Mixed regression analyses were performed to estimate exposure determinants, between- and within-worker variance of exposure, and determinants of these variances. Furthermore, we explored whether the type of waste affected the bioaerosol composition of the dust. **Results:** Endotoxin and glucan exposure levels were relatively low and comparable for waste collection and transferral, green waste composting and use of biomass in power plants. Exposure levels were 5–20 times higher in domestic waste transferral with sorting, and composting of both domestic and domestic and green waste (~ 300 – 1000 EU m^{-3} for endotoxin, and 5 – 10 $\mu g m^{-3}$ for glucan). Observed exposure exceeded Dutch occupational exposure limits at all sites. EPS-Pen/Asp exposure was detected in 20% of waste collectors and 49% of compost workers. Exposure variability within tasks was large (geometric standard deviation > 2), with smaller between-worker than within-worker variance. Type of company and waste largely explained between-worker variance (40–90%), although within companies no major task-related determinants could be established. Markers of exposure correlated moderately to strongly. Relative endotoxin and glucan content in the dust was only weakly associated with handled waste. **Conclusions:** Occupational bioaerosol exposure in the waste management chain is lowest for outdoor handling of waste and highest when waste is handled indoors. However, exposure variability is large, with greater within-worker than between-worker variance. Occupational exposure limits for organic dust and endotoxins are frequently exceeded, suggesting workers are at risk of developing adverse health effects.

Keywords: between- and within-worker variance; bioaerosol; biomass; endotoxin; $\beta(1\rightarrow3)$ -glucan; green composting; organic waste; EPS-Pen/Asp

INTRODUCTION

Waste has traditionally been disposed of by incineration or storage in landfills. However, to decrease the environmental burden associated with this, several

European countries including the Netherlands have introduced measures to reduce the total amount of waste. For this purpose separate collection of organic and non-organic household waste was introduced and incorporated throughout a large part of the society in these countries. The domestic organic waste fraction is composted in waste composting sites. In addition, composting of the so-called green waste has been encouraged. Green waste is defined as waste of

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vegetable origin produced during agriculture or the production and maintenance of private and public green areas, like lopping and mowing of parks, woods and ditch-sides. Finally, use of biomass—e.g. wood and palm kernel chips, paper sludge and animal bone meal,—as exclusive fuel source or as additional fuel source (co-firing: up to 10% of total mass besides fossil fuels) in power plants has been promoted in the Netherlands and abroad to reduce carbon dioxide emission levels (van Ree *et al.*, 2000).

Organic waste is a rich substrate for microbial growth. Therefore, handling of waste and biofuel might increase the risk of bioaerosol exposure. Bioaerosols, being airborne particulates of vegetable, animal or microbial origin, are known to lead to a wide range of health effects, as recently reviewed by Douwes *et al.* (2003). In waste handling, health effects such as respiratory symptoms, systemic influenza-like symptoms and gastrointestinal symptoms have been demonstrated to be associated with bioaerosol exposure (Nersting *et al.*, 1991; Sigsgaard *et al.*, 1994; Poulsen *et al.*, 1995a,b; Zuskin *et al.*, 1996; Ivens *et al.*, 1997; Thorn *et al.*, 1998; Douwes *et al.*, 2000; Wouters *et al.*, 2002; Heldal *et al.*, 2003a,b). Infectious diseases due to organic waste handling have been reported in some case studies as well, but in general their prevalence is low (Kramer *et al.*, 1989; Allmers *et al.*, 2000). Exposure to bacteria, especially exposure to bacterial endotoxins, is a classic and well-known cause of respiratory symptoms due to non-allergic airway inflammation (Rylander and Jacobs, 1997). Fungi are presumed to elicit allergic and non-allergic inflammatory reactions. The latter could be related to $\beta(1\rightarrow3)$ -glucans, cell wall components from most fungi (Rylander

et al., 1992; Fogelmark *et al.*, 1994; Eduard *et al.*, 2001).

The overall organic waste recycling and management chain as it developed over recent years includes several worksites with a potential of increased bioaerosol exposure. Five groups can be identified (Fig. 1): (i) waste collectors, (ii) employees at waste transferral and transport companies, (iii) workers in organic household waste composting facilities, (iv) workers in green waste composting and (v) workers in power plants where biomass is used as biofuel. To date, several studies have focused on bioaerosol exposure levels in four of these five groups at risk. Some have addressed the issue of waste collectors (Breum *et al.*, 1996; Ivens *et al.*, 1997, 1999; Nielsen *et al.*, 1997, 2000; Thorn *et al.*, 1998; Bünger *et al.*, 2000; Wouters *et al.*, 2002; Heldal *et al.*, 2003a,b), showing moderate to high bioaerosol levels. Much higher levels were found in studies on organic household waste composting (van Tongeren *et al.*, 1997; Bünger *et al.*, 2000; Douwes *et al.*, 2000). No data are available on green composting sites and only limited data on waste transferral sites (van Tongeren *et al.*, 1997). In addition, we previously showed that markers of microbial exposure in house dust were increased in homes with indoor storage of organic household waste (Wouters *et al.*, 2000). The studies mentioned above have, however, not always focused on the same bioaerosol components. Some measured viable bacteria and/or fungal spores, others have e.g. mainly focussed on airborne dust-associated fungal antigens or bacterial endotoxins. Even when the same compounds were measured, highly different extraction and analytical procedures have been applied. This complicates comparisons

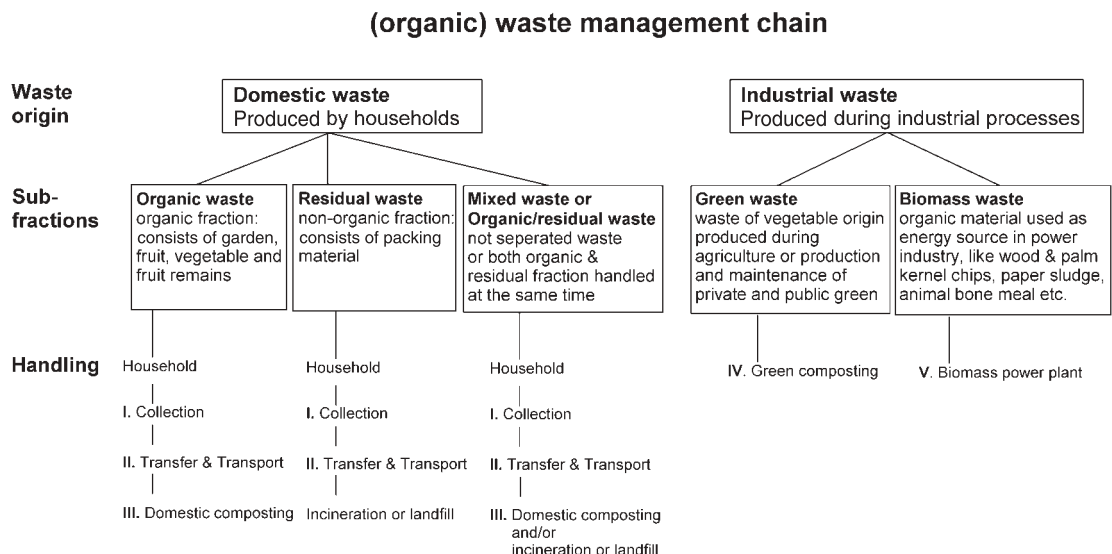


Fig. 1. Waste origin, waste fractions and chain of processes in (organic) waste removal. Indicated by roman numbers are groups suspected of increased risk for bioaerosol exposure.

between results obtained by different research groups and comparisons of exposure levels found at different sites in the waste management chain.

In the last decade, we have conducted a series of studies in a number of relatively small waste handling sites (risk sites I–V, Fig. 1). In these studies, which comprised in total over 450 personal exposure measurements, essentially the same exposure assessment procedures were applied. Exposure to inhalable dust, bacterial endotoxins and fungal $\beta(1\rightarrow3)$ -glucans—known or probable inducers of airway inflammation—were determined. Furthermore, we explored the feasibility of determining airborne levels of extracellular polysaccharides of *Penicillium* and *Aspergillus* species (EPS-*Pen/Asp*), previously shown to be a good marker for common fungal exposure if measured in settled house dust (Douwes *et al.*, 1999; Chew *et al.*, 2001). EPS has no known pathogenic role in inflammatory or allergic reactions to fungal components. Instead, EPS is considered a quantitative marker for fungal biomass, which may be more specific than $\beta(1\rightarrow3)$ -glucans as the latter might be derived from plant material as well.

In the current paper we compare levels of bioaerosol exposures in the whole waste management chain. Basic descriptive exposure analyses of some studies have been reported previously (van Tongeren *et al.*, 1997; Douwes *et al.*, 2000; Wouters *et al.*, 2002). In addition, we investigated variability in exposure over time within workers (day-to-day variance) and between workers. Finally, we explored both determinants of exposure and determinants of within- and between-worker exposure variance, and investigated whether the relative amount of endotoxin and glucan per gram of dust differed between the waste management companies.

METHODS

Definitions

Definitions of waste exposure categories, distinguished according to the origin, organic content and the waste handling processes, are summarized in Fig. 1.

Description of studies

Waste collection study: Study A. In June till September 1997, a study was conducted among domestic waste collectors of the municipal waste collecting facilities of four Dutch cities. Exposure data of the subpopulation ($n = 57$ collectors) that participated in the health effects study have been published previously (Wouters *et al.*, 2002). Presented now are the exposure data for the total population ($n = 78$ collectors). Waste is transferred into the scoop of compactor trucks. Most commonly this is performed mechanically in case the waste is offered in containers, but is

also performed by hand in case the waste is presented in small containers or plastic bags. Collected waste consisted of either separate residual and organic fractions or non-separated mixed waste. In a minority of cases other types of waste, like paper or bulk/rubbish waste, were collected. Collectors were grouped according to their main task on the day of exposure measurement into drivers, loaders and drivers/loaders (a combined task of driving and loading) on the working day. Repeated measurements were collected at the beginning (Monday/Tuesday) and at the end of the week (Thursday/Friday) for a period of 2 weeks, resulting in up to four repeated measurement per subject.

Waste transferral studies: Studies B1, B2 and B3.

Three waste transferral companies were investigated in May 1993 (van Tongeren *et al.*, 1997). In all companies, domestic waste, collected in trucks, was unloaded in a pit and repacked for further transportation, either by train or by trucks. In Company 1 residual domestic waste was, before being packed in truck containers, sorted either manually or mechanically by conveyer belts, sieves, etc. In Company 2, mixed domestic waste was transferred from the unloading pit into a rail wagon by means of a grabber that was controlled from an enclosed control room. In Company 3, separated residual and organic domestic waste was dumped directly from trucks into containers. Subsequently, the waste was dumped directly from the trucks at the unloading platform into rail wagons. All workers in the waste transferral units were investigated ($n = 9$, i.e. three in each company). Per subject, three repeated measurements were collected during a period of 1 week on Tuesday, Wednesday and Thursday without prior knowledge of activities.

Waste composting studies: Studies C1 and C2.

These studies involved an investigation conducted in November 1995 (Study C1) and November 1996 (Study C2) in a household organic waste composting facility (Douwes *et al.*, 2000). In this composting site all processes took place indoors in one big hall. Domestic organic waste was unloaded from the trucks in the hall. After pre-processing via shredding, sieving, metal removal etc. the waste was loaded into tunnels to be composted. The compost was removed from the tunnels into a sieve and placed outside to mature. In 1996 only bulldozers were used to transfer waste and compost, whereas in 1995 bulldozers and conveyer belts were used for this. Although all workers of the plant were investigated ($n = 15$ in 1995 and $n = 14$ in 1996), only four subjects were the same for both studies due to the high personnel turnover; therefore, these are presented as two separate studies. Both studies were conducted over a period of 4 weeks, and exposure was assessed at 2 days a week (Monday and Friday) in Study C1 and at 1 day a week (Monday) in Study C2.

Study D. In March–May 2001 a study was conducted among 13 domestic organic and green waste composting facilities; 3 domestic organic waste, 6 green waste, and 4 composting facilities where both domestic and green waste was composted. Workers were grouped into three categories based on their function description, their tasks and site where the work took place: being involved in composting of (i) domestic organic, (ii) green and (iii) both domestic and green waste. In total, 88 workers were included in the study, 48 in domestic, 30 in green and 10 in domestic and green composting. Bioaerosol exposure was determined once for all workers, and in part of the workers ($n = 22$) twice with a 1–1.5 month interval after the first measurements.

All procedures in domestic organic waste composting were performed indoors in large hangar-like buildings. Domestic organic waste was unloaded from trucks in the hall. Bulldozers and/or conveyer belts transferred waste and compost between machinery. Pre-treatment of the waste consisted of shredding, removing of metal parts, sieving and occasionally manual sorting. Waste was then transferred and loaded into the composting area: a tunnel ($n = 3$), a composting hall ($n = 3$) or a fermentation hall ($n = 1$). After composting the fresh compost was sieved and left to mature.

All procedures in green waste composting were performed outdoors or in partly covered buildings (two walls and a roof). Green waste was unloaded from trucks and stored outdoors in piles until processed. Bulldozers transferred waste between machinery. Green waste was pre-treated by shredding, afterwards mixed by the bulldozers, and placed on rows or piles to compost. In most facilities (8 of 10 facilities) the composting rows/piles were actively aerated and moved every 4–6 weeks by bulldozers. After composting, the compost was sieved and stored for maturation.

Use of biomass as biofuel in power production study: Study E. At the end of 2001 and the beginning of 2002 we measured bioaerosol exposure in four power plants using biomass as a fuel in the power generating process. Exposure was determined twice within a 1.5-week period. Three plants used biomass (paper pulp, wood, animal bone meal etc.) in addition to coal, and one used biomass exclusively, in particular wood. In addition we included one company producing biomass pellets for the adjacent power plant. In this last company, biomass was unloaded in the hall, transferred to a conveyer belt by means of a bulldozer, subsequently mixed and pressed into pellets. Covered conveyer belts transferred the pellets to the adjacent energy company. Operators of the plant spent half of their time driving the bulldozer and the other half doing maintenance and cleaning.

In the coal-fired power plants, biomass was mixed with coal by dispersing biomass onto the conveyer belt that transported coal to the storage bunkers. In the wood-fired power plant, wood was received and unloaded onto a conveyer belt. From the storage bunkers biomass enriched coal and/or wood was transferred to the ovens. Except for loading the fuel (both coal and biomass) the process was largely automated. Fuel loading was performed by using bulldozers, bobcats or cranes.

Exposure measurements

In all studies full-shift personal inhalable dust (defined as the mass fraction of total airborne particles that is inhaled through the nose and mouth) was sampled according to the CEN and ISO particle size selective sampling conventions (ISO, 1992; CEN, 1993). Mean sampling time over the different studies ranged from 7.5 to 8.3 h of sampling. Sampling was performed using Gillian portable constant-flow pumps at a flow rate of 2.0 l min^{-1} in combination with PAS6-samplers (Studies B, C1 and C2) or 3.5 l min^{-1} in combination with GSP-samplers (Studies A, D and E) (Kenny *et al.*, 1997), with both types of samplers equipped with Whatman GF/A glass fiber filters. Dust, endotoxin and $\beta(1\rightarrow3)$ -glucan extraction and analyses were performed as described previously (Douwes *et al.*, 1995, 1996). Briefly, dust was determined gravimetrically. Extraction for endotoxin was performed in 5 ml of 0.05% (v/v) Tween-20 in pyrogen-free water, followed by heat extraction for glucan determination (Wouters *et al.*, 2000). In supernatant, levels of endotoxin were determined by the Limulus Amebocyte Lysate assay (LAL) (Douwes *et al.*, 1995) and $\beta(1\rightarrow3)$ -glucans by the inhibition Enzyme Immuno Assay (EIA) (Douwes *et al.*, 1996). EPS-Pen/Asp levels were assessed in endotoxin extracts of the waste collectors and the compost workers study of 2001 with a previously described sandwich EIA (Douwes *et al.*, 1999; Wouters *et al.*, 2000). Limits of detection (LOD) varied between studies; appropriate values corresponding to the study are expressed in the tables in the results section. Concentrations below the LOD were assigned a value of 2/3 of the detection limit of that study.

Statistical analyses

Data analyses were performed using the SAS statistical software V8.2 (SAS institute, Cary, NC). As common with exposure data, the distribution of bioaerosol exposure levels fitted a log-normal, rather than a normal, distribution; therefore, data were log-transformed before subsequent analyses. Descriptive statistics [geometric means, geometric standard deviation (GSD) and ranges] of exposure levels were calculated, stratified per study for different tasks

and different types of waste processed. Furthermore, we explored associations between exposure markers expressed in weight units per gram of sampled dust by producing descriptive statistics stratified by the type of waste. Probabilities of non-compliance with occupational exposure limits were calculated (CEN, 1992).

Between-worker variance and day-to-day variance in exposure within workers were determined by applying mixed effects models, with worker identity as a random factor, assuming correlation between exposures measured in the same worker. We assumed that any two repeated measurements of the same worker had equal correlation (a compound symmetric covariance structure), as the sometimes limited number of repeated measurements within workers did not allow to evaluate other dependence structures as described by Peretz *et al.* (2002). Between- and within-worker variances were assumed to be equal across groups and the between-worker and within-worker variance components were estimated by using a restricted maximum likelihood method. Determinants of exposure and the effect of determinants of exposure on the between- and within-worker exposure variance were investigated as fixed factors (Rappaport *et al.*, 1999; Peretz *et al.*, 2002). The mixed-effects models is specified by the following expression:

$$Y_{ij} = \mu_y + \beta_1 + \dots + \beta_p + \gamma_i + \varepsilon_{ij},$$

for $i = 1, \dots, k$ (workers) and $j = 1, \dots, n_i$ (repetitions of the i th worker), where Y_{ij} is the log-transformed exposure level. In this model, μ_y represents an overall intercept for the group that corresponds to mean background exposure (log-transformed); β_1, \dots, β_p are fixed effects; γ_i is the random effect of the i th worker; and ε_{ij} is the random effect of the j th measurement effect of the i th worker. The assumption is that γ_i and ε_{ij} are each normally distributed and mutually independent, with mean of 0 and variances of σ_B^2 and σ_W^2 , the 'B' and 'W' subscripts are used to indicate that these variance components represent variance between workers and within workers, respectively. The estimates of σ_B^2 and σ_W^2 are presented as $B S_y^2$ and $w S_y^2$. To model the influence of exposure determinants on the exposure levels they were considered as fixed effects in the above model (β_1, \dots, β_p), and differences between predicted population means of fixed effects were tested for.

RESULTS

Overall geometric mean exposure levels in domestic waste collection were 0.6 mg m^{-3} for inhalable dust, 40.2 EU m^{-3} for endotoxin and $1.22 \text{ } \mu\text{g m}^{-3}$ for $\beta(1\rightarrow3)$ -glucans (Table 1). Exposure levels showed large variation (GSD ranging from 1.6

to 6.0; Table 1). In crude stratified analyses, only task, and not the type of waste, was associated with exposure levels (Table 1). Univariate mixed regression analysis confirmed that level of exposure was determined mainly by task and collecting regime, whereas type of truck, container, and sort of waste were only weakly associated. Being a driver on the day of the exposure measurement was associated with lower exposure levels than being a driver/loader or loader (0.58 and 0.67 times lower dust levels, 0.48 and 0.62 times lower endotoxin levels and 0.60 times lower glucan levels, $P \leq 0.05$). Collecting waste once a week resulted in higher exposure levels than collecting waste once every fortnight (1.77 times higher for dust, 1.82 times higher for endotoxin and 1.51 times higher for glucan; $P < 0.05$). The reason for this could not be established, as collection frequency and collection techniques were closely linked not allowing the discrimination of responsible factors.

Exposure levels during waste transferral are summarized in Table 2. During unloading of waste from trucks into rail wagons (Studies B2 and B3), dust and endotoxin levels were similar or slightly higher when compared with the levels in waste collection (Fig. 2). Type of waste that was unloaded did not affect the exposure levels (data not shown). In contrast, dust and endotoxin levels were much higher at sites where waste was sorted and repacked before transferral (Study B1).

Exposure levels of compost workers are presented in Table 3. In domestic organic waste composting the inhalable dust exposure levels were moderate, endotoxin exposure levels were high and comparable to or higher than in the sorting and transfer plant, and glucan levels were higher than in waste collection (Table 3; Fig. 2). Levels in green organic waste composting were low, and workers who participated in both green and domestic organic waste composting had intermediate exposures (Table 3). In all composting sites the range in exposure levels is large (GSD predominately >2.0). Job task and type of waste that was handled was a significant determinant of exposure with levels being lowest for office workers and highest for operators ($P < 0.05$).

Exposure levels in power plants (Table 4) also varied considerably, with large variation between and within job tasks (GSDs up to 15). Dust levels in the pellet producing company were high, and endotoxin and glucan levels were comparable to those in domestic composting sites. In wood and coal biomass power plants mean dust exposure levels were comparable to those in domestic waste composting, whereas endotoxin and glucan levels were in general lower and comparable to those in the waste collectors (Table 4).

In Fig. 2, an overview of inhalable dust and endotoxin exposure levels is presented for the whole waste management chain. Levels were compared with the

Table 1. Bioaerosol exposure levels in waste collection: risk site I of the organic waste management chain, overall and grouped per type of waste and task

Study	Type of waste	Task ^a	Inhalable dust (mg m ⁻³)			Endotoxin (EU m ⁻³)			Glucan (µg m ⁻³)					
			N ^b	GM ^b	(GSD) ^b	Min–Max ^b	N ^b	GM ^b	(GSD) ^b	Min–Max ^b	N ^b	GM ^b	(GSD) ^b	Min–Max ^b
A: Domestic waste collectors	All	Overall	177	0.6	(2.5)	<0.2–9.1	176	40	(3.0)	<4–7182	176	1.22	(1.4)	<0.26–52.5
	Residual	Driver	17	0.3	(2.2)	<0.2–1.5	17	30	(2.3)	8–172	16	0.84	(3.1)	<0.26–5.95
		Loader	27	0.6	(3.2)	<0.2–9.1	27	49	(4.4)	<4–7182	27	1.63	(4.2)	<0.26–30.75
		Driver/Loader	9	0.3	(1.9)	<0.2–0.7	9	30	(1.9)	12–88	9	1.06	(3.3)	<0.26–5.88
Organic	Driver	19	0.4	(2.1)	<0.2–2.2	19	20	(2.5)	<4–69	19	0.86	(2.8)	<0.26–3.35	
	Loader	32	0.7	(2.4)	<0.2–8.9	32	48	(2.2)	16–257	31	1.47	(3.5)	<0.26–12.24	
	Driver/Loader	18	0.5	(2.2)	<0.2–3.6	18	33	(3.3)	9–422	18	0.95	(4.2)	<0.26–14.77	
	Driver	8	0.6	(1.6)	0.4–1.7	8	41	(2.1)	15–131	8	1.43	(2.7)	<0.26–4.79	
Residual and organic or mixed	Loader	26	0.8	(2.0)	0.2–5.2	25	66	(3.6)	9–2279	26	1.60	(3.3)	<0.26–14.89	
	Driver/Loader	8	1.1	(3.2)	0.3–5.0	8	52	(2.8)	11–226	8	2.18	(4.5)	<0.26–24.82	
	Driver	1	0.3			1	16			1	0.33			
Other	Loader	2	1.9	(3.2)	0.8–4.3	2	51	(1.7)	36–73	2	0.60	(6.0)	<0.26–2.13	
	Driver/Loader	9	0.5	(2.1)	<0.2–1.3	9	34	(2.7)	8–159	9	2.02	(4.5)	0.39–52.48	

^aDriver = waste collector whose only task is driving the truck; Loader = waste collector whose only task is loading the waste in the compactor truck; Driver/Loader = waste collector who within a work shift alternately drives the truck and loads waste.

^bNumber of measurements (N), geometric mean (GM), geometric standard deviation (GSD), minimum (Min) and maximum (Max).

Table 2. Bioaerosol exposure levels in waste transferral: risk site II of the organic waste management chain, grouped per study and task

Study	Type of waste	Task ^a	Inhalable dust (mg m ⁻³)				Endotoxin (EU m ⁻³)			
			N ^b	GM ^b	(GSD) ^b	Min–Max ^b	N ^b	GM ^b	(GSD) ^b	Min–Max ^b
B1: Sorting and transfer	Residual	Manual pre-sorting	3	8.3	(3.7)	2.5–33.4	3	520	(3.7)	195–3536
		Driver lift truck/Operator	3	6.1	(1.6)	4.2–10.3	3	320	(1.3)	287–354
		Supervisor/Operator	3	7.3	(1.3)	5.4–9.3	3	290	(2.2)	159–684
B2: Transfer	Mixed	Grabber controller	3	1.4	(1.6)	0.9–2.3	3	71	(1.9)	37–137
		Supervisor weighing-bridge	3	0.5	(1.6)	<0.3–0.7	3	30	(1.4)	23–41
		Operator	3	1.7	(3.1)	0.5–3.4	3	61	(2.3)	24–121
B3: Tranfer	Residual and organic	Supervisor/Operator	2	2.7	(4.5)	0.9–7.9	2	36	(1.8)	24–53
		Operator wagon	6	1.2	(3.1)	<0.3–7.2	6	48	(2.3)	16–130

^aManual pre-sorting = person in waste arrival hall manually sorting bulk waste from other waste; Driver lift truck/Operator = person partly driving fork lift truck and partly inspecting operation of conveyer belts, sieves etc.; Supervisor/Operator = person partly involved in administrative tasks and partly in inspecting operation of conveyer belts, sieves etc.; Grabber controller = person in enclosed control room of grabber; Supervisor weighing-bridge = person in charge of weighing and administration of this; Operator = person involved in closing up the rail wagons and cleaning; Supervisor = person involved in maintenance and administrative tasks; Operator = person involved in opening and closing rail wagons and cleaning of the waste pit.

^bNumber of measurements (N), geometric mean (GM), geometric standard deviation (GSD), minimum (Min) and maximum (Max).

Dutch occupational exposure limit for nuisance dust of 10 mg m⁻³ and to the proposed health based occupational exposure limit for endotoxin of 50 EU m⁻³ (DECOS, 1998), and the temporary legal limit of 200 EU m⁻³ implemented on 1 January 2003 (Douwes *et al.*, 2003). Exposure levels of endotoxin were in almost all of the occupational waste management sites non-compliant with these occupational standards. Probabilities of non-compliance with the standard of 50 and 200 EU m⁻³, respectively, were 45 and 10% in waste collection, 25 and 5% in green waste composting, 100 and 33% in green & organic waste composting, 85 and 54% in domestic waste composting, and 40 and 14% in power plants. In contrast, nuisance dust standards were only incidentally exceeded with probabilities of non-compliance being 0.6% in waste collection, 1% in domestic waste composting and 6% in biofuel power production.

To explore the use of airborne EPS-*Asp/Pen* as a more specific marker of personal fungal exposure, EPS-*Asp/Pen* levels were determined in filter extracts of the waste collectors and compost workers study performed in 2001 (Studies A and D, respectively). EPS-*Pen/Asp* was detectable (>81 EPS units m⁻³) in only 20% of personal samples of waste collectors, and the samples with levels >LOD were not associated with the type of waste collected (data not shown). In the study of compost workers, filter extracts were tested for EPS two times more diluted and the LOD was therefore correspondingly higher (174 EPS units m⁻³) than in the waste collectors study. Nonetheless, a higher number (49%) of samples with detectable EPS was found in the compost industry showing in general higher levels of EPS in compost

workers than waste collectors. Like other markers of bioaerosol exposure, EPS was least frequently detectable and lowest for green waste compost workers (11% > LOD), higher in domestic organic composting (68% > LOD) and most frequently found (80% > LOD) in both types of waste composting. EPS levels were moderately correlated with dust (Spearman $R = 0.35$, $P < 0.01$) and endotoxin ($R = 0.41$, $P < 0.01$), whereas no correlation was seen with glucan levels ($R = 0.09$). Correlation coefficients improved when only samples with detectable levels of both components were taken into account ($R = 0.58$ for dust, $R = 0.74$ for endotoxin and $R = 0.28$ for glucan). The association between the different exposure markers was much better for compost workers than for waste collectors, while the latter, due to their larger number, largely determined overall correlations. In contrast, less distinct correlations for compost workers and waste collectors between dust, endotoxin and glucan levels were found (data not shown).

The overall day-to-day variance within workers in inhalable dust, endotoxin and glucan exposure was generally larger or equal to the between-worker variance (Table 5, overall). When expressed as GSD, defined as $e^{(\sqrt{\text{variance component}})}$ (Rappaport, 1991), GSDs for the within-worker variance ranged from 2.0 to 3.9 and for the between-worker variance from 1.3 to 5.2, indicating large differences in exposure between and within workers. The total variance (between plus within-worker variance) was larger for endotoxin and glucan than for dust exposure. Moreover, in power plants and domestic and green composting exposure levels of dust and more specifically endotoxin and glucan levels showed more variability

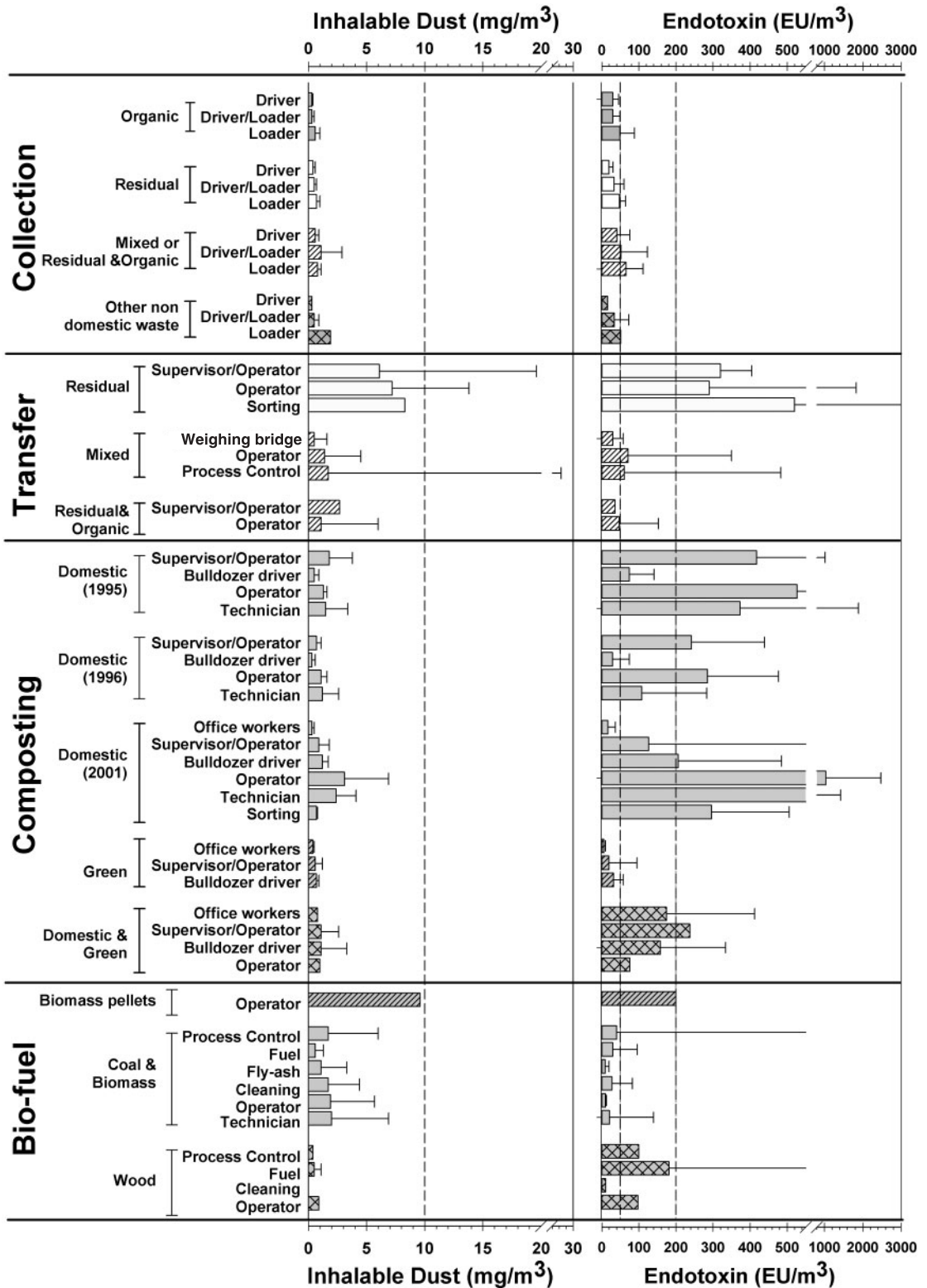


Fig. 2. Geometric mean (+ population based 95% upper confidence interval limit) of inhalable dust and endotoxin exposure levels in the organic waste management chain, grouped per waste handling site, job task, type of waste and study year (for task description see Tables 1–4). Dashed lines indicate Dutch exposure limits for dust and endotoxin (10 mg m⁻³ for inhalable dust and 50 and 200 EU m⁻³ for endotoxin), respectively.

Table 3. Bioaerosol exposure levels in domestic and green organic waste composting: risk sites III and IV of the organic waste management chain, grouped per study, type of waste and task

Study	Type of waste	Task ^a	Inhalable dust (mg m ⁻³)			Endotoxin (EU m ⁻³)			Glucan (µg m ⁻³)					
			N ^b	GM ^b	(GSD) ^b	Min-Max ^b	N ^b	GM ^b	(GSD) ^b	Min-Max ^b	N ^b	GM ^b	(GSD) ^b	Min-Max ^b
C1: Domestic waste composting	Domestic organic	Bulldozer operator	18	0.5	(3.1)	<0.3-12.2	14	75	(3.0)	<3-357	14	0.54	(3.7)	<0.15-4.83
		Technician	6	1.5	(2.2)	0.7-7.3	5	373	(3.7)	141-3544	5	4.85	(5.0)	1.03-53.23
		Supervisor/Operator	11	1.8	(3.0)	0.5-22.8	9	418	(3.2)	107-1678	9	4.28	(2.0)	1.40-10.38
		Operator	42	1.3	(2.1)	<0.3-5.3	34	527	(1.7)	220-1712	32	3.62	(2.4)	<0.15-13.18
C2: Domestic waste composting	Domestic organic	Bulldozer operator	11	0.3	(2.6)	<0.3-5.67	11	29	(4.1)	7-1314	11	0.36	(2.1)	<0.15-1.44
		Technician	6	1.2	(2.1)	0.5-2.9	6	108	(2.5)	24-299	6	4.44	(2.2)	2.00-14.12
		Supervisor/Operator	10	0.7	(1.9)	<0.3-1.6	10	242	(2.3)	60-842	10	2.14	(2.9)	<0.15-5.68
		Operator	20	1.1	(2.1)	<0.3-4.3	20	285	(3.0)	25-5965	20	3.80	(1.9)	1.05-11.96
D: Domestic and green waste composting	Domestic organic	Office worker	7	0.3	(1.6)	0.1-0.5	7	17	(2.3)	3-36	7	<0.6	(-)	<0.6-<0.4
		Bulldozer operator	18	1.2	(2.1)	0.5-4.7	19	206	(5.9)	7-8669	19	1.76	(5.0)	<0.6-25.61
		Technician	8	2.4	(1.9)	1.2-7.6	8	661	(2.5)	166-1729	8	3.43	(2.6)	1.58-16.96
		Supervisor/Operator	7	0.9	(2.1)	0.4-3.7	7	127	(5.0)	16-1509	7	0.67	(2.9)	<0.6-6.67
Green organic	Green organic	Operator	14	3.1	(4.0)	0.6-130.7	15	1038	(4.8)	129-37043	13	4.93	(6.2)	<0.6-206.6
		Manual pre-sorter	4	0.7	(1.1)	0.7-0.8	4	296	(1.4)	205-471	4	0.73	(1.6)	<0.6-1.34
		Office worker	7	0.4	(1.4)	0.2-0.6	7	6	(1.8)	4-15	8	<0.6	(-)	<0.6-<0.6
		Bulldozer operator	25	0.7	(1.7)	0.4-2.4	25	32	(4.3)	<3-345	26	0.53	(1.7)	<0.6-2.85
Domestic & Green	Domestic & Green	Supervisor/Operator	5	0.6	(1.7)	0.2-0.9	5	20	(3.5)	7-161	5	0.48	(1.5)	<0.6-0.95
		Office worker	2	0.8	(1.5)	0.6-1.1	2	175	(1.1)	168-183	3	0.52	(1.3)	<0.6-0.62
		Bulldozer operator	4	1.1	(2.0)	0.5-2.0	4	158	(1.6)	106-248	4	1.05	(2.6)	<0.6-3.85
		Supervisor/Operator	2	1.1	(1.1)	1.0-1.2	2	238	(1.5)	177-321	2	1.46	(1.5)	1.11-1.91
		Operator	1	1.0		1	76			1	0.93			

^aBulldozer operator = driving bulldozer; Technician = mechanical and/or electric engineer; Supervisor = subject partly involved in administrative tasks and partly in operating activities;

Operator = person inspecting operation of conveyor belts, sieves etc.; Manual pre-sorter = person sorting waste passing by on conveyor belt.

^bNumber of measurements (N), geometric mean (GM), geometric standard deviation (GSD), minimum (Min) and maximum (Max).

Table 4. Bioaerosol exposure levels in use of biomass in power production: risk site V of the organic waste management chain, grouped per study-site and task

Study	Type of (power) plant	Task ^a	Inhalable dust (mg m ⁻³)			Endotoxin (EU m ⁻³)			Glucan (µg m ⁻³)					
			N	GM	(GSD)	Min–Max ^b	N	GM	(GSD)	Min–Max ^b	N	GM	(GSD)	Min–Max ^b
E: Biomass in power plants	Biomass pellet prod.	Operator	2	9.6	(1.6)	6.8–13.3	2	200	(3.0)	91–437	2	12.07	(2.2)	6.8–21.3
		Cleaner	10	1.7	(3.8)	0.2–13.4	10	28	(4.6)	<3–673	10	1.39	(3.6)	<1.0–23.2
	Coal + biomass	Fly-ash	5	1.1	(2.4)	0.5–3.4	5	9	(1.9)	3–17	5	4.79	(9.7)	<1.0–166.6
		Fuel operator	12	0.6	(3.4)	0.1–4.7	12	30	(6.2)	<3.0–2104	12	1.82	(5.4)	<1.0–97.8
		Operator	4	1.9	(2.0)	0.8–3.4	4	10	(1.2)	9–13	4	<1.0		<1.0–<1.0
		Process control	6	1.7	(3.3)	0.4–10.8	6	40	(14.6)	3.1–1424	6	2.90	(6.9)	<1.0–50.8
Wood	Technician	7	2.0	(3.8)	0.3–12.0	7	21	(7.8)	<3–380	8	1.09	(2.0)	<1.0–3.2	
	Cleaner	1	<0.03			1	11			1	<1.0			
	Fuel operator	4	0.5	(1.6)	0.3–1.0	4	182	(2.5)	60–433	4	1.54	(2.8)	<1.0–5.2	
	Operator	1	0.9			1	98			1	290.9			
	Process control	2	0.4	(13.2)	0.1–2.3	2	100	(8.1)	23–438	2	2.50	(6.5)	<1.0–9.3	

^aTechnician = mechanical or electric engineer; Cleaner = person cleaning the power plant, including compressed air cleaning; Process control = person in control room, performs check-up rounds in the plant and first repairs; Fuel operator = person mainly outdoors checking unloading of fuel and loading of fuel onto the conveyor belts by shovel or crane; Operator = person in charge of transport of coal/wood into the oven, small repairs of mills, remove of bottom-ash and fly-ash and some cleaning; Fly-ash = person in charge of fly-ash bunkers including occasional sampling.

^bNumber of measurements (N), geometric mean (GM), geometric standard deviation (GSD), minimum (Min) and maximum (Max).

Table 5. Between- and within-worker variance component estimates for the one way random effects (worker only) and mixed-effects models for exposure to inhalable dust, endotoxin and glucans among workers in the organic waste management chain

	Inhalable dust		Endotoxin		Glucan	
	Within	Between	Within	Between	Within	Between
Study A: Domestic waste collectors						
Worker only	0.49	0.33	1.05	0.17	1.55	0.09
Plant	0.48	0.27	1.02	0.10	1.49	0.01
Task	0.47	0.29	0.95	0.20	1.52	0.08
Type of waste	0.49	0.30	1.04	0.16	1.55	0.09
Collection regime	0.50	0.25	1.04	0.12	1.57	0.04
Type of container	0.49	0.31	1.05	0.18	1.42	0.10
Type of truck	0.49	0.31	1.05	0.18	1.41	0.12
Type of waste + task	0.47	0.27	0.96	0.18	1.55	0.08
Study C1: Domestic organic waste composting						
Worker only	0.70	0.30	0.69	0.58	1.11	0.64
Task	0.70	0.15	0.64	ne ^a	1.08	ne ^a
Study C2: Domestic organic waste composting						
Worker only	0.60	0.24	1.15	0.97	0.71	0.88
Task	0.54	ne ^a	1.09	0.19	0.58	ne ^a
Study D: Domestic organic and green waste composting						
Worker only	0.45	0.42	1.23	2.72	0.53	1.64
Plant	0.44	0.31	1.12	1.34	0.53	1.11
Type of waste	0.45	0.34	1.14	1.53	0.54	1.31
Task	0.42	0.16	1.28	1.29	0.54	1.10
Type of waste + Task	0.43	0.14	1.16	0.81	0.55	0.97
Study E: Use of biomass in power production						
Worker only	1.42	0.51	1.32	1.94	1.85	0.82
Plant	1.27	0.14	1.43	1.19	2.32	ne ^a
Task	1.54	0.29	1.31	2.08	1.80	0.93
Type of material (wood or coal)	1.27	0.43	1.41	1.71	1.75	0.95

^ane = not estimable as the between-worker variance was estimated to be zero or negative.

than in the other sites. No clear determinants of day-to-day variability in exposure were identified, since models including determinants of exposure, such as task, type of waste and company, showed only minimal changes in within-worker variance (Table 5). Between-worker variance was strongly reduced by including determinants of exposure as fixed effects (range in explained between worker variance 10–90%; Table 5). With the exception of Study D, the determinant company best explained between-worker exposure variability, whereas other determinants such as task, type of waste, and collection regime had a less pronounced effect. In Study D, a combination of type of waste and task best described differences between persons in exposure (40–70% of between-worker variance was explained).

Finally, we explored whether type of waste affected the bioaerosol composition of the dust (Table 6). In waste collectors the relative amount of endotoxin and glucan per mg of dust was slightly higher during collection of domestic organic waste compared with collection of residual waste ($P < 0.10$; Table 6).

In composting facilities, endotoxin and glucan amounts in dust were higher for domestic and domestic/green organic waste composting than for green composting ($P < 0.05$; Table 6). However, ranges and GSDs in bioaerosol composition of dust were large, indicating large variability in dust composition.

DISCUSSION

This paper gives an overview of personal inhalable dust, endotoxin and $\beta(1\rightarrow3)$ -glucan exposure levels during collection, transferral, and composting of domestic waste, composting of green waste, and use of waste-derived biomass as biofuel in power production. Endotoxin and glucan levels were relatively low in those tasks where people worked outside and where waste was not extensively disturbed. Exposure levels were 5–20 times higher when waste was handled indoors and/or extensively agitated, e.g. when sorting before transferral, and in domestic

Table 6. Association between exposure estimates among the organic waste management chain, overall or stratified by type of waste handled. Figures represent the geometric mean (geometric standard deviation) and range of exposure ratios

Study: Type of waste	Endotoxin/Dust (EU mg ⁻¹)			Glucan/Dust (µg mg ⁻¹)		
	GM	GSD	Min–Max	GM	GSD	Min–Max
Study A: Domestic waste collectors						
Overall	69.7	(2.76)	0.4–3671	2.21	(3.05)	0.04–40.56
Domestic organic waste	84.3 ^a	(4.13)	0.4–3671	2.63	(3.33)	0.19–22.13
Domestic residual waste	59.6	(2.18)	3.9–974	1.97	(2.77)	0.27–19.32
Mixed domestic organic and residual waste	71.8	(2.26)	19.1–1599	2.09	(2.57)	0.21–14.16
Other domestic waste	60.5	(2.20)	8.4–192	2.53	(5.50)	0.04–40.56
Study C1: Domestic organic waste composting						
Overall	277.3	(2.51)	17.3–1525	2.17	(2.66)	0.05–16.22
Study C2: Domestic organic waste composting						
Overall	138.7	(3.09)	8.7–1383	2.27	(2.80)	0.03–17.53
Study D: Domestic organic and green waste composting						
Overall	101.4	(3.66)	5.1–1095	1.04	(1.87)	0.24–5.80
Domestic organic waste	186.9*	(2.83)	15.6–1095	1.27*	(1.99)	0.36–5.80
Green organic waste	34.9	(3.00)	5.05–213	0.78	(1.52)	0.24–1.86
Domestic and green organic waste	157.4*	(1.81)	55.1–308	0.95	(1.60)	0.42–1.94
Study E: Use of biomass in power production						
Overall	28.4	(4.73)	0.95–727	2.48	(5.16)	0.19–336.9

^a*P* = 0.06 domestic organic waste versus domestic residual waste in mixed effect analysis testing differences in population means.

**P* < 0.001 versus green organic waste in mixed effect analysis testing differences in population means.

organic waste and domestic and green waste composting.

Although highly comparable, the analytical techniques used in the various studies were not completely identical; two dust samplers (PAS-6 and GSP) were applied. The GSP sampler has been used in the later studies since it resembles inhalable dust conventions better at higher wind speed levels (Kenny *et al.*, 1997). Within each reported study, one type of sampler was applied and therefore within- and between-worker variance estimates were not affected. A recent EU research project investigated the sampling performance of these and other personal inhalable dust samplers in several work environments, showing good correlations and comparable dust levels for the different samplers (Kromhout *et al.*, in press). Additional analyses of the dust constituents in PAS-6 and GSP samples from the above mentioned EU study collected at a waste composting site ($n = 2 \times 7$) showed good correlations not only for dust, but also for endotoxin and glucan levels, although, absolute levels were somewhat different (~10% higher dust levels, equal endotoxin levels and 30% lower glucan levels with GSP sampling). Due to the small number of samples no firm conclusions can be drawn. However, relative ranking for worksites and job tasks over waste management sites would not have been affected, since the GSP sampler, which slightly underestimates glucan levels, has been applied in both low and high exposed environments.

Different lots of reagents for endotoxin and glucan analyses were used in the various studies described in this paper. Milton *et al.* (1997) previously described that use of different lots of LAL in endotoxin analyses may be a source of variation, possibly resulting in a factor of 2–3 difference in endotoxin concentrations. Nevertheless, results of the studies in domestic organic waste composting appeared quite comparable during the years, it thus seems unlikely that variation in results due to differences in LAL lots would have affected exposure ranking in the waste management chain. Reagents in the glucan analyses also varied, since different batches of affinity-purified rabbit IgG anti-glucan antibodies (produced in our own laboratory) and commercially purchased secondary reagents (peroxidase-labeled horse or swine anti-rabbit IgG) were used. Although these changes resulted in variation in the limit of detection (Tables 1–5), the average glucan levels in domestic waste composting studies were relatively constant, which suggests that the glucan EIA analyses did not significantly change over the years.

The proposed occupational endotoxin exposure limit of 50 EU m⁻³ by the Dutch expert committee on occupational safety and health (DECOS, 1998) and of 200 EU m⁻³ by the social economic council (Douwes *et al.*, 2003) were frequently exceeded in all sites of the waste management chain, whereas exposure limits for nuisance dust were only occasionally exceeded. Even with an exposure limit of 4 mg m⁻³, as suggested for organic dust in the animal

feed industry (Smid *et al.*, 1992), probabilities of non-compliance would be low: <1% for waste collection, green and mixed domestic waste composting, 7% for domestic waste composting and 14% for biofuel power plants. This suggests that in waste handling adverse health effects due to microbial exposures might occur in the absence of high dust levels. Any comparisons with other studies should be made with caution because of the lack of standardized methods. We therefore do not compare exposure levels with previous studies during waste management. On the other hand, it must be noted that exposure levels in waste handling are a factor 10–100 lower than previously reported endotoxin exposure in agricultural industries, such as pig and poultry farming, as summarized by Jacobs (1997).

Bioaerosol exposure is inherent to waste handling, but application of exposure control measures, such as local exhaust ventilation and encasing of conveyer belts, might reduce exposure levels, especially indoors. To date, such methods have hardly been applied. Only for bulldozer drivers control measures, by means of over pressurized cabins equipped with dust filters at the inlet, were regularly applied. However, a person's behavior (opening cabin door or window) and inadequate or lack of maintenance of filters are likely to result in only a limited effect. To comply with exposure limits, a reduction in exposure levels of a factor at least 2–10 is needed for the 200 EU m⁻³ limit and 8–40 for the 50 EU m⁻³ limit.

This is the first study reporting on airborne EPS-*Pen/Asp* exposure levels in occupational environments. Although sensitivity of the assay was not entirely adequate, we were able to detect these novel genus specific markers of fungal exposure in many personal dust samples. It is worth exploring whether sensitivity and prevalence of positive samples can be increased by applying ELISA amplification techniques as described for allergens (Renstrom *et al.*, 1997). EPS levels in airborne dust followed generally a similar pattern of exposure as other investigated microbial exposure markers. This has been described previously for house dust (Wouters *et al.*, 2000). Although EPS has no known pathogenic role in allergenic or inflammatory effects to fungi, EPS levels in house dust have been associated with respiratory health effects (Douwes *et al.*, 1999). Even so, Eduard *et al.* (2001) showed an association between EPS and health symptoms, although total number of fungi was better associated with health symptoms than both glucan and EPS levels.

In most previous studies, major determinants of exposure could not be established. Studies that tried to explain differences in exposure levels by type of waste, job tasks or type of truck also showed only a weak association between exposure and these factors (Heldal *et al.*, 1997; Nielsen *et al.*, 1997). The fact that many of the exposure determinants, such as type

of container, waste, truck and collection regime, clustered within companies makes it difficult to study exposure determinants independently, since data were not collected in all companies at the same day, or with all systems in use at the same day. Furthermore, most of the workers in waste management work outdoors, which leads to highly variable exposure levels. Only in experimentally designed studies, where other factors can be controlled for, the effects of waste type and technical systems on bioaerosol exposure can be appropriately assessed. To date, there is only one experimental study that showed lower exposure levels for waste collection trucks equipped with a mechanical exhaust ventilation system (Breum *et al.*, 1996).

Our study is the first to assess between- and within-worker variance of bioaerosol exposure in waste management, more specifically of endotoxin and $\beta(1\rightarrow3)$ -glucan exposure. In general, between-worker variance was equal to or smaller than within-worker variance (Table 5), suggesting that day-to-day differences in exposures were more prominent than differences in mean exposures between workers. Within-worker variance could not be explained by most determinants of exposure in these studies since these determinants did not change over time, as described previously by others (Peretz *et al.*, 2002). Systematic between-worker differences in exposure were associated with determinants such as job title and type of waste processed. The strongest association was, however, found with company, which suggests a major impact of factors that could not be specified in this study, but differ between companies, and are probably associated with differences in technology and working procedures. Although between-worker variance could largely be explained, remaining between-worker variance was still considerable. Rappaport (1991) defined a homogeneously exposed group as a group in which 95% of the individual mean exposures lie within a 2-fold range. Assuming a log-normal distribution of the exposure, the definition requires the between-worker variance to be ≤ 0.03 . Based upon this definition exposure in all waste management categories was not homogeneous across workers in a group, which is not uncommon in occupational exposure assessment (Rappaport 1991; Kromhout *et al.*, 1993; Preller *et al.*, 1995). Interestingly, between- and within-worker variance in endotoxin and glucan exposure (within a 100-fold range in both variances) was larger than the variance in dust exposure [within a 10-fold range in between-worker variance and a 25-fold range in within-worker variance (except for Study E)], and is in agreement with the observed high variability in biological activity of the dust (Table 6). This biological variability might also be the major factor explaining the large day-to-day variability in exposure, in addition to working in outdoor

conditions, which was another major factor determining day-to-day variability.

In conclusion, exposure levels in the organic waste chain vary widely between various waste management sites. Highest exposure levels are found in those jobs in which waste is intensively disturbed and/or handled indoors. In the highest exposure categories, mean values exceeded Dutch occupational exposure limits, suggesting that at all sites workers are at risk of developing adverse health effects. However, exposure levels at all waste sites showed large variability, with exposure levels varying more over time within workers than between workers. In addition, exposure variability in endotoxin and glucan levels was generally larger than for variability in dust exposure. This implicates that in these industries more and repeated measurements are needed to assess exposure precisely.

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