Ann. Occup. Hyg., Vol. 52, No. 5, pp. 303–316, 2008

doi:10.1093/annhyg/men024

Variability in Endotoxin Exposure Levels and Consequences for Exposure Assessment

SUZANNE SPAAN[1,2]*, JODY SCHINKEL[2], INGE M. WOUTERS[1], LIESBETH PRELLER[2], ERIK TIELEMANS[2], EVELYN TJOE NIJ[2] and DICK HEEDERIK[1]

[1]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, PO Box 80178, 3508 TD Utrecht, The Netherlands and; [2]Business Unit Quality & Safety, division Food and Chemical Risk Analysis, TNO Quality of Life, Zeist, The Netherlands

Received 23 October 2007; in final form 20 April 2008; published online 31 May 2008

Objectives: Workers in many industries are exposed to endotoxins, which may cause adverse health effects. In exposure assessment, information about exposure variability is essential. However, variability in exposure has rarely been investigated for biological agents and more specifically for endotoxin. Therefore, variance components and determinants of exposure were studied in a large database with >2000 endotoxin measurements.

Methods: Data from 10 individual studies were combined to create a database with 2010 personal inhalable dust and endotoxin measurements, of which 1650 were repeated measurements. Exposure groups were defined based on job codes. Between- and within-worker variance components were estimated for different grouping strategies, and determinants of exposure were studied using mixed effects models.

Results: Inhalable dust and endotoxin exposure levels are summarized for 46 industries and 4 broadly defined sectors. The between-worker variability exceeded the within-worker variability overall and within sectors and subsectors, and variance components were larger for endotoxin than for dust. Between-worker variability also exceeded within-worker variability in nearly half of the exposure groups based upon industries or job code within industries for endotoxin exposure and in 10% of the groups for dust exposure. Among other things, dustiness of the process, contact with animals, bulk production, presence of plant material or a cyclic process appeared as determinants of exposure, which largely explained the between-worker variability.

Conclusions: Exposure groups were much less homogeneous for endotoxin exposure than for dust exposure. This is distinctly different than for chemical exposure. Large variability in measured exposure levels is inherent to endotoxin exposure, which is caused in part by determinants that influence growth of microorganisms. These findings have major consequences for the design of future occupational intervention and epidemiological studies. The measurement effort needs to be greater than exposure assessment for chemical agents which demonstrate lower exposure variability, especially when evaluating endotoxin exposure for compliance testing. The established determinants of exposure give direction for potential exposure control, although more information about determinants of day-to-day variability in exposure is still needed to be able to effectively control endotoxin exposure.

Keywords: endotoxin exposure; exposure assessment; variance components

INTRODUCTION

Organic dust, defined as airborne particulates of vegetable, animal or microbial origin, is known to be associated with respiratory symptoms. Workers in various industries are exposed to organic dusts (Rylander and Jacobs, 1997). Endotoxins are well-known contaminants of organic dust and a major causative agent for respiratory effects (Lacey, 1994; Rylander, 1994). Endotoxin is a component of the outer cell wall of Gram-negative bacteria, and commonly present in a variety of occupational environments. Airborne endotoxins are related to the occurrence of these bacteria. Lysis after cell death results in release of endotoxins into the environment.

*Author to whom correspondence should be addressed.
Tel: +31-30-253-9474; fax: +31-30-253-9499;
e-mail: S.Spaan@uu.nl
The free full text of this article can be found in the online version of this issue.

Inhalation is thought to be the major route of exposure and is associated with respiratory and systemic inflammatory responses, both acute and chronic (Rietschel *et al.*, 1994; Rylander and Jacobs, 1997; Liebers *et al.*, 2006). There are also indications that environmental endotoxin exposure has a potential protective effect on the risk of atopic sensitization in childhood, and possibly also in adults working with high endotoxin exposures (von Mutius *et al.*, 2000; Liu, 2002; Portengen *et al.*, 2005; Smit *et al.*, 2008).

Gram-negative bacteria are ubiquitous in the environment. Their growth is dependent upon many factors, including the presence of a substrate for nutrients, favourable water activity and temperature. Aerosolization and distribution of particles are necessary conditions for exposure. Consequently, exposure to endotoxin is highly variable. The inherent exponential amplification of living microorganisms probably contributes to the environmental variability in exposure. Furthermore, possible growth of organisms on the filter or in the extract after sampling, reaction of organisms or their products with the assay or agglomeration in the solution may cause even more variability in the results from analysis of biological agents and thus the measured exposure. This is in contrast with exposure to chemical agents, where such factors do not play a role.

Traditionally, measured exposure for compliance testing used to be assessed by sampling workers at one point in time during a worst-case exposure scenario. Yet, information about sources of between- and within-person exposure variability are needed in order to accurately assess overexposure and exceedance (Tornero-Velez *et al.*, 1997) or effectively advise on determinants of exposure and control measures. For this purpose, randomly collected repeated exposure measurements from a representative subset of workers in an occupational group are essential (Symanski *et al.*, 2006). The first studies to evaluate exposure variability predominantly focused at airborne exposure to chemical agents (Kromhout *et al.*, 1993; Rappaport *et al.*, 1993). After that, other studies also explored exposure variance components, in more industries, for other substances and for different routes of exposure (Kromhout and Vermeulen, 2001; Symanski and Greeson, 2002; Symanski *et al.*, 2006).

Variability in exposure to biological agents has rarely been investigated, although the total exposed population is substantial. However, knowledge about variability in exposure is a necessary requirement and vital starting point for (future) measurement campaigns. Insight into components of exposure variability and underlying determinants may also influence the design of intervention (Lazovich *et al.*, 2002) and epidemiologic studies (Tielemans *et al.*, 1998). Variance components can be used in compliance testing as well (Rappaport *et al.*, 1995).

We constructed a database containing >2000 full-shift endotoxin measurements to inform research in the area of compliance testing, intervention studies and epidemiology. As far as we know, this is the first large endotoxin exposure database that has been composed and analysed for variance components and determinants of exposure. The specific aims of this study were to

(i) give an overview of exposure to inhalable dust and endotoxin in a range of industries using comparable measurement protocols,
(ii) investigate determinants of exposure, to get insight in factors that influence exposure across sectors and industries and
(iii) study between- and within-worker variance components as a basis for a protocol for a measurement strategy for endotoxin exposure assessment like those that exist for chemical agents, as described elsewhere (Spaan *et al.*, 2007).

METHODS

Development of database

The study was performed using a database with personal inhalable exposure data collected in 10 studies, performed over the years by the Institute for Risk Assessment Sciences (Utrecht, The Netherlands; former Department of Environmental and Occupational Health at Wageningen University) and TNO (Zeist, The Netherlands). The studies were conducted over the years 1991–2006 and most results have been published in open literature (Preller *et al.*, 1995a; Smit *et al.*, 2005; Smit *et al.*, 2006; Spaan *et al.*, 2006; Wouters *et al.*, 2006). The original data sets of these studies were made available for this current investigation. Details of the exposure assessment of the studies have been described previously. Briefly, the measurements were performed with 25-mm Teflon filters (PAS-6 sampler, one study, 17% of measurements), 25-mm glass fibre filters (PAS-6 sampler, one study, 5%) or 37-mm glass fibre filters (GSP sampler, eight studies, 78%). After sampling, filters were stored at −20°C until extraction. The filters were extracted in pyrogen-free water with (nine studies, 83%) or without (one study, 17%) 0.05% Tween-20 and stored at −20°C until endotoxin analysis. All extracts were analysed with the kinetic chromogenic Limulus amoebocyte lysate (LAL) assay in one laboratory. In all studies LAL reagents from the same producer were used (BioWhitakker).

The original data sets included 2147 measurements. After excluding task-based and stationary measurements and measurements without known endotoxin concentration, 2010 personal inhalable measurements were available, gathered from >1000 workers from 317 factories in 46 industries (see

Table 2). Of these measurements, 1650 were repeated measurements from 730 workers.

The industries were classified into the following four sectors based on similarities regarding the kind of process, materials/products being used or the manufactured end product:

(i) Waste management/treatment: all industries that handle any kind of waste product, including domestic waste.
(ii) Grains, seeds and legumes processing: the primary production of grains, seeds, legumes etc., as well as the (industrial) processing of these materials into half-products and consumption goods.
(iii) Horticulture: the indoor (greenhouse) and outdoor culturing and trade of vegetables, flowers and plants, as well as the (industrial) processing of these products.
(iv) Animal production: the primary production (farms) and industrial processing (abattoirs, etc.) of animals or animal products.

If applicable, a further subdivision within sectors was made based on similarities between industries within a sector; these groups of industries within sectors were called subsectors.

Determinants as listed in Table 1 were either already identified at time of the study or identified afterwards by consulting the primary investigators. Nonetheless, not all information was available or could be assigned afterwards, e.g. descriptive components like tasks performed or products handled during the measurement, due to the diverse nature and original purposes of the underlying studies.

The structural relationship of the data was organized in several levels, namely:

(i) measurements within workers,
(ii) workers within jobs,
(iii) jobs within factories,
(iv) factories within industries and
(v) industries within subsectors and sectors.

In some industries, measurements were performed in several factories with analogue processes (composting, domestic waste collection, sewage treatment and pig farms), but in most industries only one or two factories were included (Table 2). The measurements were grouped on job code level within industries for statistical purposes, leading to a total of 147 groups.

Statistical analysis

Data were analysed with SAS statistical software (version 8e; SAS Institute, Cary, NC, USA). Levels of exposure were lognormally transformed before statistical analysis. Exposure levels were calculated as geometric mean (GM) with geometric standard deviation (GSD) for each sector, subsector and industry. Spearman correlations were calculated between inhalable dust and endotoxin concentrations.

Between-worker and day-to-day (within-worker) variance in exposure were determined by applying mixed effects models. Worker identity was introduced as a random factor in order to correct for possible correlation between repeated measurements in the same worker. Any two repeated measurements of the same worker were assumed to have equal correlation (a compound symmetric covariance structure). Between- and within-variance components were estimated by using a restricted maximum likelihood method. In addition, a multilevel approach was used to investigate the manner in which the variability in exposure was distributed over the structural relationship levels in the database by adding those levels as random effects. For statistical purposes, here the maximum likelihood method was used.

Determinants of exposure were identified, and the effect of these determinants on the between- and within-worker exposure variance was investigated by introducing them as fixed effects (Rappaport *et al.*, 1999; Peretz *et al.*, 2002). For this analysis, only part of the data set was used (1757 of 2010 measurements). Measurements with missing data for one or more of the determinants of interest were removed from the data set to ensure stability of the analysis. A forward stepwise procedure was followed, applying the χ^2 goodness of fit test (based on -2RLL, restricted log likelihood) for all determinants. Only determinants with a significant χ^2 test were added to the model. Separate models were constructed for endotoxin and inhalable dust exposure. Graphical analyses of residuals were performed to evaluate assumptions of homoscedasticity.

Between- and within-worker variance components were estimated for each exposure group (job code within industry). Exposure groups with less than two workers measured twice were excluded from the analysis. As a result, 66 exposure groups were excluded from this analysis. The ratios of the 97.5th and 2.5th percentiles of the variance components of the lognormally distributed endotoxin and dust exposures were estimated for each exposure group. These ratios provide information regarding the ranges of exposures found between workers ($_{\text{between-worker}}R_{0.95}$) and within workers from day-to-day ($_{\text{within-worker}}R_{0.95}$) (Rappaport, 1991). The cumulative distributions (%) of these ratios were plotted both for endotoxin and dust.

RESULTS

Specification of data

The mean sampling time of the measurements in the database was 7.2 h; only 1% of the observations had a sampling time of <4 h. Furthermore, 90 and 62 measurements were below the limit of detection (LOD) for endotoxin and dust, respectively. These were given a value of two-thirds the LOD for endotoxin

Table 1. Information about variables in the database

Variable	Description
Survey	Study the measurements originates from
Sector	Description of sector
Industry	Description of industry
Factory	Unique indication
Worker	Unique number
Job	Description of job
Job code	Classification of job
Tasks	Tasks performed (with duration) during measurement
Products	Products handled during measurement
Date	Date of measurement
Exposure concentration	Measured concentration (dust, endotoxin or glucan)
Detection limit	Below (−1) or at or above (0) the detection limit (for endotoxin)
Units	Unit of measurement (mg m^{-3}, EU m^{-3} or μg m^{-3})
Analysis	Kind of analysis used to measure exposure
Sampling time	Duration of measurement (in min)
Flow	Mean flow during sampling
Sampling equipment	Kind of sampling head used: GSP (=1), PAS6 (=2)
Filter type	Kind of filter used: 37-mm glass fibre, 25-mm glass fibre and 25-mm Teflon
Use of Tween during extraction	No (0) or yes (1)
Sample of workers	Non-random (=0), random (=1), random within jobs (=2) and everybody (=3)
Sample of days	Non-random, season (=0), random (=1), fixed days (=2) and every day (=3)
Environment	Working environment mainly: outside (=1), inside (=2), outside and inside (=3)
Season	Winter (=1), spring (=2), summer (=3) and autumn (=4)
Mechanical ventilation	Absent (=0) or present (=1)
Natural ventilation	Absent (=0) or present (=1)
Local exhaust ventilation (LEV)	Absent (=0) or present (=1)
Use personal protective equipment (PPE)	Use of personal protective equipment: no (=0) or yes (=1)
Wastewater	Absent (=0) or present (=1)
Process water	Absent (=0) or present (=1)
Recycling process water	Absent (=0) or present (=1)
Source of exposure	Local (=0) or general (=1)
Mobility of source	Stationary (=0) or mobile (=1)
Mobility of worker	Stationary (=0) or mobile (=1)
Process	Intermittent (=0) or continuous (=1) (coded based on the work day)
Cyclic process	Absent (= 0) or present (= 1) (coded based at the process cycle as a whole)
Length of process	Short (=0) or long (=1)
Bulk production/processing	Absent (=0) or present (=1)
Dusty process	Absent (=0) or present (=1)
Exposure pattern	Continued (=0) or variable (=1)
Industrial process	Absent (=0) or present (=1)
Microbial growth in process	Absent (=0) or present (=1)
Plant material	Absent (=0) or present (=1)
Formation (watery) aerosol	Absent (=0) or present (=1)
Damp environment	Absent (=0) or present (=1)
Faeces (human or animal)	Absent (=0) or present (=1)
Contact with living animals	Absent (=0) or present (=1) (at worker level)
Animals	Absent (=0) or present (=1) (at factory level)

and dust of the particular study from which they originated. For 75 measurements, the dust level was missing. In none of the industries were there >25% of the observations below the LOD. Glucan exposures were assessed in some of the studies, but insufficient data were available for inclusion in this analysis.

Table 2. Characteristics of the endotoxin database—number of measurements, mean exposure levels for endotoxin (EU m^{-3}), inhalable dust (mg m^{-3}) and endotoxin per mg dust (EU mg^{-1}) and correlations between endotoxin and dust for subsectors and industries

Sector					Endotoxin (EU m^{-3})		Dust (mg m^{-3})		EU mg^{-1} dust	
Industry	C[a]	*N* (repeat)[b]	*K* (repeat)[c]	*G*[d]	GM (GSD)	Range	GM (GSD)	Range	GM (GSD)	*r*[e]
Overall (total database)	317	2010 (1653)	1089 (732)	147	160 (8.6)	0.6–19 1400	0.77 (4.3)	nd–131	220 (4.4)	0.75*
Waste treatment and management	65	951 (751)	482 (282)	29	48.0 (4.9)	0.6–37 000	0.40 (3.7)	nd–131	122 (4.1)	0.56*
Domestic waste collection	4	179 (162)	79 (53)	2	40.2 (3.0)	2.4–7180	0.58 (2.5)	0.15–9.1	69.7 (2.8)	0.63*
Mushroom compost preparation	2	41 (34)	24 (17)	4	225 (3.2)	14.1–2430	0.50 (2.3)	0.08–2.6	452 (2.4)	0.62*
Composting	13	215 (150)	115 (50)	7	155 (5.7)	1.8–37 000	0.98 (2.6)	0.13–131	155 (3.4)	0.75*
Wood power plant	1	8 (6)	5 (3)	4	102 (3.9)	11.4–438	0.33 (4.9)	0.02–2.34	306 (1.9)	0.93*
Coal and biomass power plant	3	48 (18)	39 (9)	7	26.5 (5.7)	2.2–2100	1.37 (3.5)	0.09–13.4	19.4 (4.0)	0.57*
Sewage treatment plant	42	460 (381)	220 (141)	5	27.2 (3.7)	0.6–2090	0.17 (3.2)	nd–23.5	150 (4.3)	0.38*
Grains, seeds and legumes processing	26	351 (292)	202 (145)	61	633 (8.6)	2.3–149 000	1.47 (4.8)	nd–102	431 (4.6)	0.71*
Primary production	3	15 (6)	12 (3)	4	2700 (4.6)	95.5–41 200	2.47 (4.3)	0.27–56.5	1092 (2.9)	0.74*
Potato cultivation	1	2 (—)	2 (—)	1	314 (5.4)	95.5–1030	1.97 (3.9)	0.76–5.2	159 (1.4)	—
Grain harvest	1	3 (2)	2 (1)	1	2100 (2.5)	1030–5790	0.53 (2.1)	0.27–1.2	3990 (1.6)	0.50
Flax culture and processing	1	10 (4)	8 (2)	2	4470 (3.7)	685–41 200	4.11 (4.0)	0.59–56.5	1090 (1.7)	0.85*
(Industrial) processing	19	262 (220)	151 (109)	46	831 (8.3)	9.1–131 000	1.76 (4.6)	nd–102	473 (4.5)	0.70*
Grain trans-shipment and derivates	1	19 (14)	12 (7)	3	2150 (9.0)	113–131 000	6.71 (5.1)	0.77–98.5	321 (3.4)	0.79*
Cereal seed	1	2 (2)	1 (1)	2	3560 (2.9)	1690–7470	4.05 (1.5)	3.00–5.5	879 (1.9)	—
Grass/corn seed	2	28 (20)	18 (10)	4	1160 (6.7)	9.1–51 400	1.44 (5.0)	0.13–98.1	804 (2.8)	0.89*
Grass-drying plant	1	5 (4)	3 (2)	3	2700 (6.2)	179–20 200	3.71 (4.0)	0.52–18.3	780 (1.6)	1.00*
Grass seed	3	27 (24)	14 (11)	4	5470 (4.0)	222–79 900	3.37 (3.9)	0.11–34.7	1620 (2.2)	0.72*
Vegetable seed	3	39 (34)	22 (17)	4	770 (6.4)	25.6–42 200	0.96 (3.4)	0.14–14.1	803 (3.5)	0.74*
Grinding industry	1	17 (16)	9 (8)	5	2810 (4.1)	257–35 900	3.50 (2.2)	1.07–16.9	803 (5.9)	−0.38
Corn processing	1	14 (8)	10 (4)	5	710 (7.3)	35.9–30 700	7.45 (3.6)	0.75–41.7	95.3 (4.8)	0.54*
Meal/flour tillage and processing	1	16 (12)	10 (6)	4	281 (7.7)	19.1–28 200	1.40 (3.1)	0.20–7.3	202 (3.4)	0.89*
Animal feed industry	4	87 (78)	48 (39)	10	270 (7.0)	14.4–80 500	1.10 (4.5)	nd–102	245 (3.6)	0.78*
Malting plant	1	8 (8)	4 (4)	2	3730 (4.3)	291–20 000	0.73 (1.5)	0.41–1.3	5130 (3.0)	0.88*
Processing for consumption	4	74 (66)	41 (33)	11	181 (6.6)	2.3–149 000	0.70 (4.7)	nd–79.6	257 (4.7)	0.50*
Industrial bakery	1	12 (10)	7 (5)	2	49.2 (7.4)	2.3–3030	1.23 (3.0)	0.27–11.2	40.1 (5.5)	0.46
Coffee roasting and tea trading	1	19 (16)	11 (8)	2	138 (3.4)	12.4–2030	0.69 (2.5)	0.17–2.7	198 (3.1)	0.52*
Rice hulling plant	1	16 (16)	8 (8)	4	1110 (7.6)	95.1–149 000	3.06 (6.0)	0.34–79.6	362 (1.9)	0.94*
Sugar production (sugar beets)	1	27 (24)	15 (12)	3	134 (4.0)	9.4–2520	0.23 (2.7)	nd–1.3	576 (3.9)	0.33[#]
Horticulture	19	250 (216)	142 (108)	38	162 (7.6)	1.6–191 400	0.67 (3.7)	nd–35.1	242 (3.9)	0.60*
Greenhouse	9	120 (106)	67 (53)	18	106 (4.3)	1.6–4130	0.52 (2.7)	nd–11.4	204 (3.1)	0.65*
Flower bulbs nursery	2	21 (16)	13 (8)	2	565 (3.9)	9.7–4130	1.35 (2.3)	0.28–11.4	419 (3.0)	0.66*

Table 2. *Continued*

Sector					Endotoxin (EU m^{-3})		Dust (mg m^{-3})		EU mg^{-1} dust	
Industry	C[a]	*N* (repeat)[b]	*K* (repeat)[c]	*G*[d]	GM (GSD)	Range	GM (GSD)	Range	GM (GSD)	*r*[e]
Mushroom nursery/growing	1	17 (16)	9 (8)	4	80.8 (4.0)	2.9–1350	0.22 (4.2)	nd–0.85	375 (5.6)	0.13
Cucumber and paprika nursery	1	14 (10)	9 (5)	2	157 (2.2)	36.1–650	0.57 (2.1)	0.25–2.4	275 (2.0)	0.77*
Pot-plants nursery (*Ficus*)	1	8 (8)	4 (4)	2	47.6 (6.7)	1.6–1490	0.31 (2.5)	0.14–2.4	156 (3.6)	0.74*
Cut flowers nursery/growing	2	31 (28)	17 (14)	2	39.5 (2.7)	4.9–326	0.31 (1.5)	0.11–0.68	127 (2.1)	0.76*
Tomatoes nursery	1	10 (10)	5 (5)	3	68.7 (2.5)	13.8–342	0.83 (1.7)	0.44–1.9	83.2 (1.8)	0.75*
Chicory nursery/growing	1	19 (18)	10 (9)	3	136 (2.6)	35.4–769	0.82 (1.6)	0.37–2.0	165 (2.0)	0.67*
Outdoor	4	50 (44)	28 (22)	4	107 (2.5)	8.6–449	0.89 (2.4)	0.10–9.2	120 (2.5)	0.52*
Hardy nursery stock and trading	2	29 (28)	15 (14)	3	123 (2.1)	18.6–347	1.33 (2.1)	0.33–9.2	92.6 (2.1)	0.59*
Gardening company	2	21 (16)	13 (8)	1	88.7 (3.1)	8.6–449	0.52 (2.0)	0.10–1.7	171 (3.0)	0.46*
(Industrial) processing	4	44 (34)	27 (17)	10	60.9 (4.9)	4.9–1200	0.25 (1.9)	nd–1.5	241 (5.9)	−0.03
Dried subtropical fruit	1	15 (10)	10 (5)	3	19.4 (2.3)	4.9–148	0.40 (1.8)	0.18–1.5	49.0 (2.0)	0.63*
Vegetable and fruit processing	2	20 (20)	10 (10)	5	177 (4.2)	11.1–1200	0.23 (1.4)	0.13–0.34	758 (4.0)	0.27
Vegetable slicing plant	1	9 (4)	7 (2)	2	38.8 (3.9)	8.5–594	0.14 (2.4)	nd–0.48	268 (6.8)	0.00
Trade	2	36 (32)	20 (16)	6	4000 (9.8)	107–191 400	3.50 (5.4)	0.18–35.0	1145 (2.2)	0.94*
Flower bulb trade	1	16 (16)	8 (8)	4	388 (1.8)	107–1220	0.59 (1.9)	0.18–2.7	653 (1.7)	0.71*
Onion trade	1	20 (16)	12 (8)	2	25 900 (2.7)	4030–191 400	14.5 (1.5)	6.67–35.0	1790 (2.0)	0.77*
Animal production	207	458 (394)	261 (197)	19	681 (5.2)	2.0–19 500	1.78 (2.8)	nd–26.6	383 (3.1)	0.67*
Primary production	202	377 (332)	211 (166)	3	1190 (2.4)	62.2–19 500	2.40 (1.9)	0.36–26.6	496 (2.2)	0.52*
Dairy farming	2	12 (12)	6 (6)	1	788 (3.6)	62.2–3860	1.35 (1.8)	0.36–2.7	584 (2.7)	0.41
Poultry farm	3	9 (6)	6 (3)	1	1750 (2.8)	360–8120	4.59 (2.0)	1.58–13.6	381 (2.5)	0.70*
Pig farm	197	356 (314)	199 (157)	1	1190 (2.4)	73.0–19 500	2.40 (1.9)	0.36–26.6	496 (2.1)	0.52*
(Industrial) processing	5	81 (62)	50 (31)	16	51.0 (6.8)	2.0–6230	0.44 (3.7)	nd–21.3	116 (5.0)	0.48*
Calf abattoir	1	12 (12)	6 (6)	2	119 (11.8)	2.6–3480	0.23 (4.9)	nd–2.1	512 (5.0)	0.70*
Poultry abattoir	1	14 (12)	8 (6)	4	308 (7.0)	26.5–6230	1.47 (5.3)	0.22–21.3	209 (1.6)	0.94*
Cow/cattle abattoir	1	19 (14)	12 (7)	4	30.5 (5.2)	2.0–816.8	0.27 (1.9)	0.07–1.9	113 (5.4)	0.09
Pig/swine abattoir	1	16 (12)	10 (6)	2	27.5 (3.4)	2.4–218.5	0.31 (1.6)	0.15–0.61	89.1 (3.1)	0.38
Meat processing	1	20 (12)	14 (6)	4	23.3 (3.6)	3.0–1420	0.59 (3.3)	0.14–10.9	39.6 (4.9)	0.21

nd, below detection limit.
[a]Number of companies/factories per industry included in the database.
[b]Number of measurements (number of repeated measurements).
[c]Number of workers (number of workers with repeated measurements).
[d]Number of jobs measured.
[e]correlation coefficient (*r*) for inhalable dust and endotoxin exposure.
*$P < 0.05$; #$0.05 < P < 0.10$.

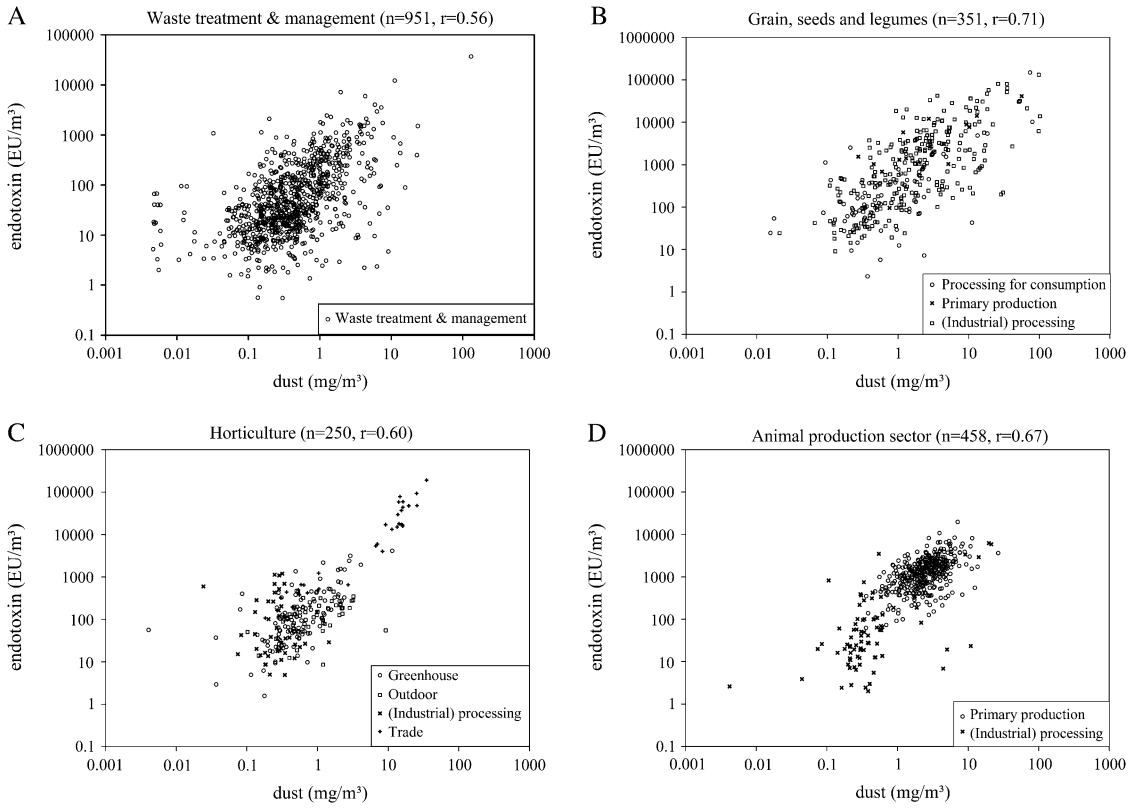

Fig. 1. Dust and endotoxin levels per sector (A–D) on a log scale, with number of measurements and correlation coefficient per sector.

Exposure levels

Table 2 shows endotoxin, inhalable dust and endotoxin per mg dust levels. The overall GM concentration was 160 EU m^{-3} for endotoxins and 0.8 mg m^{-3} for inhalable dust, with distinctly more spread in endotoxin exposure (GSD 8.6 for endotoxin versus 4.3 for dust). Similarly, endotoxin exposure showed generally larger variability in sectors and industries, both between workers and between days. Highest mean exposure levels for both endotoxin and dust were found in animal production. In 'grains, seeds and legumes processing' and 'animal production', distinct differences were seen between primary production (front end) and subsequent processing. In 'waste treatment and management' and 'horticulture', exposure levels were not as clearly different between subsectors, although trade in horticulture had much higher dust and endotoxin exposure levels than the other subsectors. In general, exposure in these sectors was lower than in animal production and grains, seeds and legumes processing.

Correlation analysis

Correlations between inhalable dust and endotoxin differed considerably between sectors and industries within sectors, ranging from virtually no correlation to very strong correlation (Table 2 and Fig. 1A–D). The overall correlation coefficient was high (Spearman 0.75).

Variance components

Overall, for both endotoxin and inhalable dust levels, the between-worker variability was larger than day-to-day variability. The same was seen when the data were grouped in sectors or subsectors (Table 3). This indicates that differences in exposure between workers were larger than between work shifts on different days. Exceptions were the waste treatment and management sector, outdoor horticulture and primary animal production for dust exposure, and greenhouse horticulture and primary animal production for endotoxin exposure (Table 3). The ratio of between-worker and within-worker variability was less distinct when workers were grouped in industries or in job codes within industries. Between-worker variability exceeded within-worker variability in 26 and 20 of 46 industries for endotoxin and dust, respectively. Between-worker variability exceeded day-to-day variability in 39 groups in case of endotoxin exposure and in 35 groups when looking at dust exposure in the 81 job code within-industry groups with enough repeated measurements. Both for workers grouped in industries or in job codes within industries, in almost all cases total variance was

Table 3. Between- and within-worker variance components in the database, based on log-transformed data ($n = 2010$)

	Endotoxin (EU m^{-3})		Dust (mg m^{-3})	
	BW	WW	BW	WW
Total database	3.72*	1.00*	1.44*	0.71*
Waste treatment and management	1.42*	1.13*	0.84*	0.90*
Grains, seeds and legumes processing	3.55*	1.00*	1.67*	0.79*
Primary production	1.25	1.06	1.35#	0.67#
(Industrial) processing	3.30*	1.08*	1.44*	0.86*
Processing for consumption	2.84*	0.72*	1.92*	0.52*
Horticulture	3.27*	0.96*	1.27*	0.4*
Greenhouse	0.67*	1.47*	0.40*	0.60*
Outdoor	0.47*	0.37*	0.21#	0.54#
(Industrial) processing	1.88*	0.59*	0.35*	0.17*
Trade	4.91*	0.35*	2.66*	0.16*
Animal production sector	2.04*	0.71*	0.69*	0.39*
Primary production	0.15*	0.63*	0.11*	0.32*
(Industrial) processing	2.56*	1.00*	0.91*	0.72*

BW, between-worker variance component; WW, within-worker variance component.
*$P < 0.05$; #$0.05 < P < 0.10$.

larger for endotoxin exposure than dust exposure (data not shown).

Different hierarchical levels (sector, subsector, industry, factory, job code and worker) could be distinguished in the database. To gain better insight into between- and within-worker variability, the distribution of exposure variability over these levels was investigated in the same part of the database used for the determinant analysis ($n = 1757$). Only the between-worker variance was affected taking into account the hierarchical structure of the data, whereas the within-worker variance component did not change noticeably. The between-worker variance component ($\sigma^2_{\text{between worker}} = 3.6$) broke down into a between-subsector, between-industry, between-factory, between-job (job code within industry) and between-worker component. After the between-job component was included ($\sigma^2_{\text{between job}} = 3.1$ and $\sigma^2_{\text{between worker}} = 0.4$), adding other levels like factory or industry did not significantly improve the model, although the individual variance components differed statistically significant from zero. The between-worker variance component levelled off at a value of ~0.4 for endotoxin exposure and 0.2 for dust exposure, which was clearly lower than in the worker-only model (data not shown).

Grouping workers by job codes within industries and excluding groups with less than two subjects and two repeats left 81 groups with 975 workers and 1840 measurements. For these 81 groups, median total, between- and within-worker GSDs were 3.44, 1.95 and 2.22 for endotoxin exposure and 2.53, 1.62 and 1.73 for dust exposure. In Fig. 2, the distributions of the within- and between-worker $R_{0.95}$ for endotoxin and dust exposure are shown for these 81 groups. Generally, both the between- and within-worker values of $R_{0.95}$ were larger for endotoxin than for dust. Only 21 (26%) and 22 (27%) groups had 95% of the individual mean exposures within a factor 2 ($_{\text{between-worker}}R_{0.95} \leq 2$) for endotoxin and dust, respectively. Furthermore, 54 and 40% of the groups had values of $_{\text{between-worker}}R_{0.95} > 10$, and 35 and 12% of the groups even had values of $_{\text{between-worker}}R_{0.95} > 50$ for endotoxin and dust, respectively. Part of the groups had a $_{\text{between-worker}}R_{0.95} = 1$ for both endotoxin and dust (Fig. 2). This is due to an estimation problem when calculating variance components. The between-worker variance component is estimated with a zero or negative value in case of a large within-worker GSD or few repeated measurements.

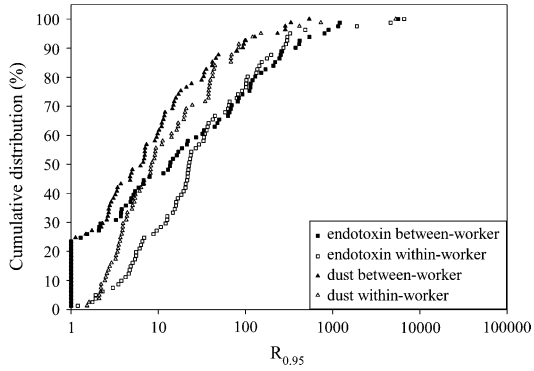

Fig. 2. Cumulative distributions of $_{\text{B}}R_{0.95}$ (closed) and $_{\text{W}}R_{0.95}$ (open) for endotoxin (squares) and dust (triangles) for 81 groups based on job code within industry.

Determinants of exposure and exposure variability

Potential determinants of exposure (see Table 1) were tested as fixed effects in a stepwise procedure to explain variability in endotoxin and dust exposure. The effect estimates for variables significantly contributing to the model are shown in Table 4. In the first model, subsector and job code were used initially to account for the influence of these two variables on exposure level. In addition to subsector and job code, a dusty process, contact with living animals and a continuous process increased both endotoxin and dust exposure, while a cyclic process and microbial growth (when the latter is an inherent part of the process) were associated with decreased exposure levels for both endotoxin and dust. Working inside was associated with increased endotoxin exposure but decreased dust exposure. Furthermore, the models for endotoxin and dust exposure consisted of different additional determinants. Presence of plant material and faeces and production in bulk were

Table 4. Relative effect of determinants of exposure on endotoxin and inhalable dust levels, in part of the data set ($n = 1757$)

Variables	Coding variables	1. Model with subsector, job code and determinants		2. Model with determinants only		3. Model with job code within industry and determinants	
		Endotoxin e^{β} (95% CI)	Inhalable dust e^{β} (95% CI)	Endotoxin e^{β} (95% CI)	Inhalable dust e^{β} (95% CI)	Endotoxin e^{β} (95% CI)	Inhalable dust e^{β} (95% CI)
Intercept		20.1 (13.2–30.7)*	0.3 (0.2–0.5)*	76.4 (52.7–111)*	0.7 (0.4–0.9)*	154 (76.7–309)*	0.7 (0.4–1.2)
Subsector	10	x	x	—	—	—	—
Job code	14	x	x	—	—	—	—
Job code within industry	124	—	—	—	—	x	x
Dusty process	Present versus absent	2.7 (1.4–5.2)*	2.3 (1.1–4.9)*	1.9 (1.3–2.8)*	2.2 (1.7–2.9)*	—	—
Contact animals	Present versus absent	7.5 (3.3–17.0)*	3.2 (1.8–5.8)*	3.8 (2.5–5.9)*	2.2 (1.6–3.0)*	—	—
Plant material	Present versus absent	1.6 (1.2–2.2)*	—	—	0.7 (0.6–0.9)*	—	—
Work environment	Inside	2.1 (1.4–3.0)*	0.7 (0.5–1.0)*	2.1 (1.4–3.0)*	—	5.5 (3.2–9.4)*	—
	Both inside and outside	1.9 (1.3–2.9)*	1.2 (0.8–1.8)	1.4 (1.0–2.1)*	—	5.7 (2.7–12.3)*	—
	Outside (reference)	1.0	1.0	1.0	—	1.0	—
Cyclic process	Present versus absent	0.2 (0.1–0.3)*	0.2 (0.2–0.3)*	—	0.4 (0.3–0.6)*	—	—
Bulk production	Present versus absent	4.0 (2.4–6.4)*	—	7.0 (4.6–10.8)*	1.4 (1.1–1.9)*	—	—
Process	Continuous versus intermittent	5.2 (3.0–9.1)*	4.3 (2.4–7.8)*	2.7 (1.8–3.9)*	2.5 (1.8–3.5)*	—	—
Microbial growth	Present versus absent	0.2 (0.1–0.4)*	0.2 (0.1–0.5)*	0.2 (0.1–0.3)*	0.3 (0.2–0.4)*	—	—
Faeces	Present versus absent	2.6 (1.6–4.1)*	—	—	—	—	—
Local exhaust ventilation (LEV)	Present versus absent	0.7 (0.5–0.9)*	—	—	—	—	—
Exposure	Variable versus continuous	—	1.7 (1.2–2.4)*	—	2.0 (1.5–2.5)*	—	1.8 (1.4–2.4)*
Cycle	Long versus short	—	3.1 (1.3–7.9)*	1.9 (1.2–3.1)*	2.5 (1.7–3.8)*	—	—
Season	Spring	—	1.5 (1.2–1.8)*	0.9 (0.7–1.1)	1.2 (1.0–1.4)	0.8 (0.5–1.1)	—
	Summer	—	0.8 (0.7–0.9)*	0.8 (0.6–0.9)*	0.8 (0.7–0.9)*	0.7 (0.5–0.8)*	—
	Autumn	—	2.0 (1.3–3.1)*	1.0 (0.7–1.6)	1.5 (1.1–2.1)*	0.4 (0.2–0.7)*	—
	Winter (reference)	—	1.0	1.0	1.0	1.0	—
Formation aerosol	Present versus absent	—	—	0.6 (0.4–0.8)*	—	—	—
Wastewater	Present versus absent	—	—	0.6 (0.4–0.8)*	0.7 (0.6–0.9)	—	0.7 (0.5–0.9)*
Industrial process	Present versus absent	—	—	0.3 (0.2–0.5)*	0.7 (0.5–0.9)	—	—
Mobility of source	Mobile versus stationary	—	—	—	0.6 (0.5–0.8)*	—	—

x, estimates not given; —, variable not in model.
*$P < 0.05$.

associated with higher endotoxin levels, whereas presence of local exhaust ventilation was associated with lower endotoxin levels. For dust exposure, intermittent exposure (versus continuous) and a prolonged (versus short) cycle were associated with higher dust levels, while season had a variable effect.

In a model with determinants only (Model 2), the outcomes were comparable to those of Model 1, with similar effect estimates. However, some determinants no longer had a significant effect on the measured endotoxin exposure (presence of plant material, faeces and local exhaust ventilation), while other determinants were included (a long work cycle, an industrial process, presence of wastewater and formation of aerosol). For dust exposure, the work environment was no longer a part of the model, while production in bulk, presence of plant material, wastewater, an industrial process and a mobile source were added (Table 4).

When job code within industry was introduced as a fixed effect (Model 3), only work environment and season remained as determinants of endotoxin exposure. As for dust exposure, exposure pattern of the worker and presence of wastewater in the process were the only determinants remaining, with their effect pointing towards the same direction as in the other models (Table 4).

Since exposure variability could be distributed over more levels than worker only, we also investigated the way determinants of endotoxin and dust exposure affected a model with both worker and job categories within an industry as random effects. For both endotoxin and dust exposure, approximately similar models were found, with effect estimates analogous to that of a model with only worker as random effect (data not shown).

Because information about some possible determinants (mechanical ventilation, recirculating process water and source of exposure) was missing for a considerable number of measurements in the database, the additional effect of these variables in the abovementioned models was tested in a subset of the data set with complete data for these variables. Addition of these variables did not result in significant additional effects (data not shown).

No clear determinants of day-to-day variability in exposure were identified. Models including determinants of exposure showed only minimal changes in within-worker variability for both endotoxin and dust. However, inclusion of the lognormally transformed dust concentration explained 3–18% of the within-worker variance in endotoxin exposure (Table 5). The between-worker variance was strongly reduced by introducing determinants of exposure. With regard to endotoxin exposure, inclusion of the variable subsector, industry or factory in the model explained 62, 78 and 78% of the between-worker variability, respectively (Table 5). Model 1 explained 80% and Model 2 explained 72% of both the endotoxin and dust exposure. Introducing job categories within an industry explained 88% of the endotoxin and dust exposure, and introducing other determinants had no substantial additional effect. In the multilevel models with worker and job code within industry as random effects, determinants mostly explained between-job variability (∼60% for both endotoxin and dust exposure) and only a little between-worker variability (∼5%) (data not shown).

Table 5. Percentage explained variance by certain variables or models, in part of the data set (n = 1757)

Model with fixed effects	Endotoxin		Inhalable dust	
	BW (%)[a]	WW (%)[a]	BW (%)[a]	WW (%)[a]
Worker only	3.64	0.97	1.38	0.67
Model with sector	2.15 (41)	0.96 (∼0)	0.91 (34)	0.67 (∼0)
Model with subsector	1.38 (62)	0.96 (∼0)	0.69 (50)	0.66 (∼0)
Model with industry	0.79 (78)	0.98 (∼0)	0.29 (79)	0.65 (3)
Model with factory	0.80 (78)	0.97 (∼0)	[a]	
Model with job code	2.82 (23)	0.96 (∼0)	0.97 (30)	0.68 (∼0)
Model with job code within sector	1.32 (64)	0.96 (∼0)	[a]	
Model with job code within industry	0.44 (88)	0.96 (∼0)	0.17 (88)	0.65 (3)
Model with dust concentration	1.12 (69)	0.94 (3)	x	
Model with subsector, job code and determinants (1)	0.69 (81)	0.96 (∼0)	0.28 (80)	0.66 (∼0)
Model with subsector, job code, determinants and dust concentration	0.41 (89)	0.80 (18)	x	
Model with determinants only (2)	1.02 (72)	0.97 (∼0)	0.38 (72)	0.67 (∼0)
Model with determinants and dust concentration	0.54 (85)	0.82 (15)	x	
Model with job code within industry and determinants (3)	0.41 (89)	0.94 (3)	0.16 (88)	0.65 (3)

BW, between-worker variance; WW, within-worker variance.
[a]Stopped because of infinite likelihood, percentage explained variance.

DISCUSSION

A database with many measurements for inhalable dust and endotoxin exposure was set up successfully. Data from 10 individual studies comprising >2000 measurements in >1000 workers were put together in a combined database. Although endotoxin exposure levels of the individual studies have been published before, this is the first study to investigate determinants of exposure levels and more importantly of exposure variability in a broad spectrum of working environments. Measurements in the database have all been collected and analysed within the same two closely collaborating research groups and analysed in one laboratory, which minimized the introduction of additional variability.

Some of the individual studies looked, among other variables, at the influence of tasks and specific characteristics of the process on exposure levels (Preller *et al.*, 1995a,b; Smit *et al.*, 2006; Wouters *et al.*, 2006). Unfortunately, this detailed information could not be gathered for all measurements in the database. Nonetheless, it can be concluded that determinants of exposure identified in this database are generally in accordance with previous results and give a more complete picture of determinants of dust and endotoxin exposure. For instance, dustiness of the process and contact with animals were found to be determinants of exposure in both the study in agricultural industries (Spaan *et al.*, 2006) and this database. The study in agricultural industries comprises 'only' a fourth of the total database, which also contains data from studies in other industries with different characteristics as well as data from studies in one or a limited set of agricultural industries. Surprisingly, the previously observed exposure determinants appeared to be of importance when taking into account other industries as well. These determinants provide information for the implementation of generic control measures to lower exposure, for instance reducing the dustiness of products or dust formation by preventing leakage from the process. The presence of determinants that are associated with aqueous media (wastewater, formation of watery aerosols and microbial growth as part of a (moist) process) seem to be associated with lower endotoxin and dust exposures.

Since job code within industry is a very specific categorization, and most of the possible determinants were classified at factory level, not many other variables added a significant effect to the model with job code within industry (Model 3). Furthermore, many of the determinants of exposure occurring in the model with subsector and job code (Model 1) also appeared in a model without subsector and job code (Model 2). This suggests that although many determinants are captured in the variable subsector, other determinants are specific enough to explain some of the differences in endotoxin and dust exposure, which enhances the reliability of the various models. The influence of variables reflecting differences in procedures between the studies (filter type, sampler type and extraction/assay medium used) was also investigated. These did not have an effect and did not change the models.

This database is a collection of individual studies that were initially not set up to be joined at a later stage, which means that they all had their own design and specific study aims. This influenced the way workers, factories and measurement days were selected and information was collected (on job code or industry level, specific or more general). The extent of detail in the information varied from almost none to elaborate information about tasks. Coding afterwards may have led to some misclassification, determinants that are left undetected and availability of information on a more general level.

Most determinants on which information was available concerned company or process characteristics and dust formation and were coded on company and sometimes worker level. These types of determinants do not vary over time. Therefore, it is not surprising that almost no day-to-day variability could be explained by these variables. Only measured dust concentration explained some variance in endotoxin exposure, but this is expected since measured dust exposure captures day-to-day variability. Furthermore, not all exposure variability could be explained by the current determinants, compared to the models with job code or industry as fixed effects, which suggests that other determinants of exposure also play a role. For a more detailed analysis of exposure determinants, more refined information at a personal level and for instance on sources of exposure and amount of microbiological growth is needed.

When investigating determinants of exposure in the total database, homogeneity of variance components over determinants is assumed (Symanski *et al.*, 2001; Weaver *et al.*, 2001; van Tongeren *et al.*, 2006). Since the variance components did not differ much between different sectors, and overall between-worker variability exceeded within-worker variability, this assumption seems reasonable. Furthermore, subsector and job code were included as fixed effects to correct for possible differences.

An important aim of this study was to analyse variance components for endotoxin and dust exposure. Overall, there was a relatively high variability in both endotoxin and inhalable dust exposure, with more variability in endotoxin levels. Furthermore, between-worker variability was generally larger than within-worker variability. This differs distinctly from the situation of exposure to chemical agents. For instance, Kromhout *et al.* found median values for total, within- and between-worker GSDs of 2.41, 2.00 and 1.43, respectively, in a database for inhalatory exposure to chemical agents (Kromhout *et al.*,

1993). A study on dermal exposure to chemicals showed similar results, with median values for the total, within- and between-worker GSDs of 2.55, 1.98 and 1.47, respectively (Kromhout and Vermeulen, 2001). The total variance for inhalable dust exposure found in our database was in the same range (median GSD_{total} 2.53), but endotoxin exposure variability was distinctly larger (median GSD_{total} 3.44). Furthermore, between-worker variance was larger as well, although there was more within-worker than between-worker exposure variability when workers were grouped by job code within industry. For both chemical exposure and endotoxin exposure, ~25% of the groups based on job code categories were homogeneous ($_{between\text{-}worker}R_{0.95} \leq 2$). However, for endotoxin 54% of the groups had a $_{between\text{-}worker}R_{0.95} > 10$ and 35% even a $_{between\text{-}worker}R_{0.95} > 50$, compared to 30 and 10% for chemical exposure, respectively (Kromhout *et al.*, 1993). In a previously published meta-analysis, day-to-day variation in exposure generally exceeded variation between workers, with exposure in the chemical industry, on average, more homogeneous than exposures in non-chemical industries. Gaseous exposures were also more homogeneous in comparison with exposures to aerosols or dermal exposures (Symanski *et al.*, 2006). This may partly be due to a greater degree of variation from day to day in factors that influence the emission of gases/vapours (temperature and pressure) compared with those factors that govern the emission of aerosols (mechanical and physical forces) and the physical and chemical characteristics of the agent that also affect variation in exposure after emission (Vincent, 1995; Mulhausen and Damiano, 1998).

Studies have shown large inter- and intra-laboratory differences for endotoxin analysis. It has been suggested that this may explain the high variability in endotoxin exposure (Linsel *et al.*, 2002; Reynolds *et al.*, 2002; Reynolds *et al.*, 2005; Chun *et al.*, 2006). However, in the studies in this paper, methods used were fairly similar and protocols only changed marginally over time. The analytical error for endotoxin, expressed as coefficient of variation (CV%), is generally <20%. This analytical error is usually part of the within-worker variance. After correction for this analytical error, the within-worker variance component would only change marginally ($GSD_{within\ worker}$ 2.4 after correction compared to 2.7 for the crude $GSD_{within\ worker}$), which points out that the analytical variance is much smaller than the variability over time. This is in accordance with Nicas *et al.*, who stated that measurement error is often small relative to exposure variability over time (Nicas *et al.*, 1991). Thus, most of the variability in endotoxin exposure is an inherent part of the true exposure to endotoxin and is presumably caused by the fact that endotoxins originate from Gram-negative bacteria, which grow and amplify. Storage conditions, contamination of process water, differences in temperature, number of microorganisms in the product (for instance differences between seasons caused by the conditions on the land during culturing and harvesting) and type of dust may all have an effect on the measured endotoxin level. When circumstances are optimal, microbiological activity in products and processes can increase exponentially and lead to an increased endotoxin exposure and increased exposure variability, both between and within workers, even over a very short period of time. The varying endotoxin content of dust confirms this observation. Exposure to chemical agents is not influenced by such factors and thus varies considerably less.

It is expected that workers who have similar jobs have more similar exposures (relative to the variation in exposure over time) than workers at the same location but with different jobs. However, lack of homogeneity in exposure in a considerable number of groups is reported before (Kromhout *et al.*, 1993; Symanski and Greeson, 2002). When bio-monitoring data were grouped on plant level, more variation among workers at the same plant than variation from day to day was found (Symanski and Greeson, 2002). The same was seen in the endotoxin data, with relatively more between-worker than within-worker variability when grouping workers at sector or industry level, but vice versa when workers were grouped on a more detailed level based on job code. Hence, to arrive at relatively homogeneous exposure groups, one should use a grouping scheme based on at least job code and perhaps an even more detailed level is needed. This information is important in designing an optimal exposure assessment approach for epidemiological studies.

The large variability in endotoxin exposure also has consequences for existing compliance strategies and the estimation of exposure for epidemiological studies. For instance, the existing CEN 689 guideline for measuring chemical components (CEN, 1995) needs to be modified to take into account the greater variability in endotoxin exposure. If the decision-making process is based on calculations of overexposure and exceedance (Tornero-Velez *et al.*, 1997), for which between- and within-worker variance components from this endotoxin database are used (Spaan *et al.*, 2007), the number of measurements available, variance around the mean exposure and selected cut-off points (e.g. ½ or ¼ of the occupational exposure limit (OEL)) determine the probability of making an accurate decision based on the number of samples taken (Leidel *et al.*, 1977; Stellingwerf, 1984). Since the exposure variability for endotoxin is large, more measurements will be needed to estimate the probability of overexposure or exceedance.

CONCLUSIONS

As far as we know, this is the first endotoxin exposure database that has been created and analysed for

variance components and determinants of exposure. Data from future studies can be added in order to get a more complete picture of occupational endotoxin and dust exposure and factors that influence exposure. Variance components give insight into the variability in endotoxin and inhalable dust exposure, with more variability overall between workers than from day to day. This differs from exposures to chemical agents. Also, the total variance in endotoxin exposure was higher than for chemical exposures, with fewer and less homogeneous exposure groups. Therefore, large variability in measured exposure levels is an inherent part of endotoxin exposure, caused by many factors that influence growth of microorganisms and the process of aerosolization. This should be taken into account when assessing endotoxin exposure. It has consequences for the design of future occupational intervention and epidemiologic studies. The variables in the database may function as a guideline for information that should be gathered. The observed determinants of exposure could be a starting point for the development of control measures, although more insight into determinants that cause day-to-day variability is needed.

FUNDING

Ministry of Social Affairs and Employment, The Netherlands.

Acknowledgements—The authors would like to acknowledge all financiers, researchers, students and laboratory workers who made the studies that are part of the database that was used in this study, in particular Lidwien Smit. Furthermore, we thank Tim Meijster and Lutzen Portengen for their assistance in the statistical analysis.

REFERENCES

CEN. (1995) 689: Workplace atmospheres—guidance for the assessment of exposure by inhalation to chemical agents for comparison with limit values and measurement strategy. Brussels, Belgium: European Committee for Standardization.

Chun DT, Bartlett K, Gordon T *et al*. (2006) History and results of the two inter-laboratory round robin endotoxin assay studies on cotton dust. Am J Ind Med; 49: 301–6.

Kromhout H, Symanski E, Rappaport SM. (1993) A comprehensive evaluation of within- and between-worker components of occupational exposure to chemical agents. Ann Occup Hyg; 37: 253–70.

Kromhout H, Vermeulen R. (2001) Temporal, personal and spatial variability in dermal exposure. Ann Occup Hyg; 45: 257–73.

Lacey J. (1994) Microorganisms in organic dust. In: Rylander R and Jacobs RR, editors. Organic dusts: exposure, effects and prevention. London: Lewis Publishers pp. 17–41.

Lazovich D, Murray DM, Brosseau LM *et al*. (2002) Sample size considerations for studies of intervention efficacy in the occupational setting. Ann Occup Hyg; 46: 219–27.

Leidel NA, Busch KA, Lynch JR. (1977) Occupational exposure sampling strategy manual. Cincinnati, OH: National Institute of Occupational Safety and Health.

Liebers V, Bruning T, Raulf-Heimsoth M. (2006) Occupational endotoxin-exposure and possible health effects on humans. Am J Ind Med; 49: 474–91.

Linsel G, Doering C, Duggal S *et al*. (2002) Ergebnisse eines Ringversuches zur Messung luftgetragener Endotoxine. VDI Ber; 1656: 329–39.

Liu AH. (2002) Endotoxin exposure in allergy and asthma: reconciling a paradox. J Allergy Clin Immunol; 109: 379–92.

Mulhausen JR, Damiano J. (1998) A strategy for assessing and managing occupational exposures. Fairfax, VA: AIHA Press.

Nicas M, Simmons BP, Spear RC. (1991) Environmental versus analytical variability in exposure measurements. Am Ind Hyg Assoc J; 52: 553–7.

Peretz C, Goren A, Smid T *et al*. (2002) Application of mixed-effects models for exposure assessment. Ann Occup Hyg; 46: 69–77.

Portengen L, Preller L, Tielen M *et al*. (2005) Endotoxin exposure and atopic sensitization in adult pig farmers. J Allergy Clin Immunol; 115: 797–802.

Preller L, Heederik D, Kromhout H *et al*. (1995a) Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modelling. Ann Occup Hyg; 39: 545–57.

Preller L, Kromhout H, Heederik D *et al*. (1995b) Modeling long-term average exposure in occupational exposure-response analysis. Scand J Work Environ Health; 21: 504–12.

Rappaport SM. (1991) Assessment of long-term exposures to toxic substances in air. Ann Occup Hyg; 35: 61–121.

Rappaport SM, Kromhout H, Symanski E. (1993) Variation of exposure between workers in homogeneous exposure groups. Am Ind Hyg Assoc J; 54: 654–62.

Rappaport SM, Lyles RH, Kupper LL. (1995) An exposure-assessments strategy accounting for within- and between-worker sources of variability. Ann Occup Hyg; 39: 469–95.

Rappaport SM, Weaver M, Taylor D *et al*. (1999) Application of mixed models to assess exposures monitored by construction workers during hot processes. Ann Occup Hyg; 43: 457–69.

Reynolds SJ, Thorne PS, Donham KJ *et al*. (2002) Comparison of endotoxin assays using agricultural dusts. Am Ind Hyg Assoc J; 63: 430–8.

Reynolds SJ, Milton DK, Heederik D *et al*. (2005) Interlaboratory evaluation of endotoxin analyses in agricultural dusts—comparison of LAL assay and mass spectrometry. J Environ Monit; 7: 1371–7.

Rietschel ET, Kirikae T, Schade FU *et al*. (1994) Bacterial endotoxin: molecular relationships of structure to activity and function. FASEB J; 8: 217–25.

Rylander R. (1994) Organic dusts—from knowledge to prevention. Scand J Work Environ Health; 20: Spec No: 116–22.

Rylander R, Jacobs RR. (1997) Endotoxins in the environment—a criteria document. Int J Occup Environ Health; 3: S1–48.

Smit LA, Spaan S, Heederik D. (2005) Endotoxin exposure and symptoms in wastewater treatment workers. Am J Ind Med; 48: 30–9.

Smit LA, Wouters IM, Hobo MM *et al*. (2006) Agricultural seed dust as a potential cause of organic dust toxic syndrome. Occup Environ Med; 63: 59–67.

Smit LA, Heederik D, Doekes G *et al*. (2008) Exposure-response analysis of allergy and respiratory symptoms in endotoxin exposed adults. Eur Respir J [epub ahead of print, Feb 2008].

Spaan S, Schinkel JM, Wouters IM *et al*. (2007) Endotoxin: from database to measurement strategy. Gefahrst Reinhalt Luft; 67: 377–83.

Spaan S, Wouters IM, Oosting I *et al*. (2006) Exposure to inhalable dust and endotoxins in agricultural industries. J Environ Monit; 8: 63–72.

Stellingwerf J. (1984) Time dependent aspects in the european communities directives on asbestos and lead. Letter to the editor. Br J Ind Med; 41: 354–6.

Symanski E, Greeson NM. (2002) Assessment of variability in biomonitoring data using a large database of biological measures of exposure. Am Ind Hyg Assoc J; 63: 390–401.

Symanski E, Sallsten G, Chan W *et al*. (2001) Heterogeneity in sources of exposure variability among groups of workers exposed to inorganic mercury. Ann Occup Hyg; 45: 677–87.

Symanski E, Maberti S, Chan W. (2006) A meta-analytic approach for characterizing the within-worker and between-worker sources of variation in occupational exposure. Ann Occup Hyg; 50: 343–57.

Tielemans E, Kupper LL, Kromhout H *et al*. (1998) Individual-based and group-based occupational exposure assessment: some equations to evaluate different strategies. Ann Occup Hyg; 42: 115–9.

Tornero-Velez R, Symanski E, Kromhout H *et al*. (1997) Compliance versus risk in assessing occupational exposures. Risk Anal; 17: 279–92.

van Tongeren M, Burstyn I, Kromhout H *et al*. (2006) Are variance components of exposure heterogeneous between time periods and factories in the European carbon black industry? Ann Occup Hyg; 50: 55–64.

Vincent JH. (1995) Aerosol science for industrial hygienists. Oxford: Pergamon, Elsevier Science Limited.

von Mutius E, Braun-Fahrlander C, Schierl R *et al*. (2000) Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy; 30: 1230–4.

Weaver MA, Kupper LL, Taylor D *et al*. (2001) Simultaneous assessment of occupational exposures from multiple worker groups. Ann Occup Hyg; 45: 525–42.

Wouters IM, Spaan S, Douwes J *et al*. (2006) Overview of personal occupational exposure levels to inhalable dust, endotoxin, beta(1–>3)-glucan and fungal extracellular polysaccharides in the waste management chain. Ann Occup Hyg; 50: 39–53.