

Scientific paper

DOI: 10.2478/10004-1254-61-2010-1996

ENDOTOXIN EXPOSURE ASSESSMENT IN WOOD-PROCESSING INDUSTRY: AIRBORNE VERSUS SETTLED DUST LEVELS

Ivana SABOLIĆ PIPINIĆ¹, Veda Marija VARNAI¹, Ružica BELJO LUČIĆ²,
Ankica ČAVLOVIĆ², Ljerka PRESTER¹, Tatjana ORCT¹, and Jelena MACAN¹

Institute for Medical Research and Occupational Health¹, Faculty of Forestry, University of Zagreb², Zagreb, Croatia

Received in August 2009

Accepted in January 2010

Wood processing is usually performed in environments with large amounts of endotoxin-rich bioaerosols that are associated with a variety of health effects. The aim of this preliminary study was to assess the relation between endotoxin levels in settled and airborne dust in wood-processing industry. Ten pairs of airborne and settled dust samples were collected in a sawmill and parquet manufacture of two wood-processing plants in Croatia. Endotoxin was assayed with a chromogenic end-point LAL (*Limulus* amoebocyte lysate) method. The results showed that endotoxin levels in airborne respirable dust were above the proposed occupational exposure limit of 125 EU m⁻³ and could be considered hazardous for the respiratory system. In settled dust they ranged between 229.7 EU mg⁻¹ and 604.3 EU mg⁻¹ and in airborne dust between 166.8 EU mg⁻¹ and 671.6 EU m⁻³, but there was no significant correlation between them (Spearman's rho=0.358, P=0.310). This study points to sawmill settled dust as endotoxin reservoir and suggests that it may add to already high exposure to airborne endotoxins associated with wood processing. Investigations of the relation between settled and airborne endotoxin levels should be continued to better understand the sources and sites of endotoxin contamination in wood-processing industry.

KEY WORDS: *airborne dust, bioaerosols, LAL test, parquet manufacture, sawmill*

Endotoxins are integral components of the outer membrane of Gram-negative bacteria with strong inflammatory properties (1). Released in the environment, they make an important, biologically active component of organic dust at home and work, and, if inhaled, induce dose-related inflammatory response (2). Occupational monitoring of endotoxin is mostly based on airborne endotoxin levels (1, 3), while measurement in settled dust is not as common in occupational settings, even though it is less expensive and less time consuming. There are indications, however, that settled dust endotoxin could be used as an indicator of indoor endotoxin exposure (4, 5).

The aim of this preliminary study was to compare settled and airborne dust endotoxin levels in wood-

processing industry measured at the same time, since such data are not available in published literature. Wood-processing industry is one of the working environments with large amounts of endotoxin-rich bioaerosols, which are associated with various health effects (1, 3).

METHODS

Sampling

Air dust and settled dust were sampled at two Croatian sawmills (A and B) and the parquet manufacture unit of sawmill B over the summer. Sawmill A processed solid beechwood (*Fagus*

silvatica L.), oakwood (*Quercus Robur* L.), and firwood (*Abies Alba* Mill.), and sawmill B, processed ashwood (*Fraxinus angustifolia* Vahl.) in addition to beechwood and oakwood. Samples were gathered during sawing and debarking of logs in both sawmills and during parquet manufacture in sawmill B. Five pairs of air dust and settled dust samples were collected in Sawmill A, three pairs in sawmill B, and two pairs in the parquet manufacture unit of sawmill B.

Airborne dust samples were collected with stationary samplers for respirable particles (Casella, Bedford, UK). The device was attached to a stand near the workplace or working machine, with the collecting positioned in the breathing area according to the HRN CEN/TR 15230:2005 standard (6). Sampler cellular filters (Whatman International Ltd., Maidstone, UK) were stored at -20 °C until extraction. Control filters were handled, stored, and analysed for endotoxin load in the same way as the sample filters.

Settled dust samples were taken from a floor area of a square metre or two in close vicinity of a corresponding air dust sampler. Each sample was collected with a brush in a clean plastic bag and stored at -20 °C until extraction. At each sampling site, air temperature, relative humidity, and airflow velocity were measured using a Kestrel® 4000 Pocket Weather™ Tracker™ (Nielsen-Kellerman Co., USA).

Endotoxin measurement

Each filter was placed in 5 mL of pyrogen-free LAL water (Endosafe, lot no. 997320659) containing 0.05 % Tween-20 (LAL-T). Dust samples were sieved and 100 mg of fine dust was extracted in 10 mL of pyrogen-free LAL-T. The extracts were centrifuged and supernatants transferred into pyrogen-free glassware at 75 °C for 20 min to avoid any possible interference (7).

Endotoxins were analysed on the day of extraction using a quantitative chromogenic end-point LAL method (*Limulus* amoebocyte lysate) at 37 °C. Glassware was rendered pyrogen-free by heating it to 180 °C for 4 h. A commercial kit for endotoxin analysis was purchased from Charles River Endosafe (Charleston, USA; lot no. Y4042CT1 and lot no. V2242CTK1). For standard, we used the *Escherichia coli* endotoxin (Endosafe; CSE lot no. ET 84092) with a potency of 4 EU ng⁻¹. Aliquots (100 µL) of reference standard, extracts, reagent blanks, and controls were added to pyrogen-free microtitre plates (Greiner Labortechnik, GmbH, Germany), and assayed in duplicate as described earlier (8). Standards were prepared as

twofold dilutions containing endotoxin concentrations from 0.015 EU mL⁻¹ to 1.2 EU mL⁻¹. Optical density was read at 405 nm on a Personal Lab 96-well microplate reader (Iason, Graz, Austria). Endotoxin concentrations are expressed as endotoxin units (EU) per milligram of dust (EU mg⁻¹) or per cubic meter of sampled air (EU m⁻³). The maximum sensitivity of the test was 0.015 EU mL⁻¹.

Statistical analysis

Nonparametric statistics was applied, because endotoxin data were not normally distributed. Descriptive statistics, testing of the difference in endotoxin values between the two sawmills (Mann-Whitney U test), and testing of the correlation between airborne and settled dust endotoxin values (Spearman correlation) were performed using statistical software Stata/SE 10.0 for Windows (StatCorp LP, TX, USA).

RESULTS

Measurements in both sawmills were performed during the summer with temperatures at sampling sites ranging from 24.4 °C to 30 °C (median 27.8 °C). Relative humidity ranged from 47 % to 56.3 % (median 51.2 %). Airflow velocity was 0 m s⁻¹ at both sawmills.

Table 1 shows endotoxin values in parallel samples of airborne and settled dust. No differences were found between the two sawmills, either for airborne dust [sawmill A: median 261.7 EU m⁻³, range: (228.1 to 617.6) EU m⁻³; sawmill B: median 280.5 EU m⁻³, range: (166.8 to 671.6) EU m⁻³; $z=0.313$, $P=0.754$] or for settled dust [sawmill A: median 474.3 EU mg⁻¹, range: (255.2 to 500.9) EU mg⁻¹; sawmill B: median 330.6 EU mg⁻¹, range: (229.7 to 604.3) EU mg⁻¹; $z=-0.104$, $P=0.917$]. The same is true for the two samples collected from parquet manufacture, and this is why we pooled data from both sawmills, including parquet manufacture, for further analysis. Endotoxin in settled and in airborne dust was relatively limited in range [(229.7 to 604.3) EU mg⁻¹ and (166.8 to 671.6) EU m⁻³, respectively] and there was no significant correlation between the two (Spearman's $\rho=0.358$, $P=0.310$).

DISCUSSION AND CONCLUSIONS

In this study, all endotoxin levels in airborne dust were above the exposure limit of 5 ng m⁻³ (50 EU

Table 1 Endotoxin levels in settled and airborne dust from sawmills

Sample pair	Type of operation	Airborne endotoxin / EU m ⁻³	Settled dust endotoxin / EU mg ⁻¹
1	sawmill A ^a	232.1	255.2
2	sawmill A ^a	228.1	292.3
3	sawmill A ^a	261.7	474.3
4	sawmill A ^a	316.6	494.9
5	sawmill A ^a	617.6	500.9
6	sawmill B ^a	232.6	558.2
7	sawmill B ^a	166.8	229.7
8	sawmill B ^a	671.6	260.2
9	parquet manufacturing unit ^b (sawmill B)	357.1	330.6
10	parquet manufacturing unit (sawmill B) ^b	280.5	604.3
All samples (sawmills and parquet)			
Median (interquartile range)		271.1 (232.1 to 357.1)	402.5 (260.2 to 500.9)
Arithmetic mean±SD		336.5±171.0	400.1±140.4

^aWet-wood processing

^bDry-wood processing

Table 2 Comparison of airborne and settled dust endotoxin levels in different environments

Environment (number of settled dust samples / number of airborne dust samples)	Settled endotoxin / EU mg ⁻¹		Airborne endotoxin / EU m ⁻³		Ratio of settled and airborne dust endotoxin average values	Reference
	Average	Range	Average	Range		
Household (93/116) ^c	96 ^a	28 to 1945	0.77 ^a	0.01 to 30.23	125	21
Household (24/23) ^d	8.7 ^a	21 to 48.0	0.43 ^a	0.05 to 3.99	20	4
Aircraft cabin (32/28) ^c	120 ^a	3.8 to 420	1.5 ^a	0.53 to 5.3	80	20
Animal houses ^e						
Dairy cattle (11/22)	365 ^b	51 to 1032	3.1 ^b	0.3 to 61	118	
Beef cattle (3/6)	1079 ^b	95 to 1114	10.2 ^b	0.9 to 31	106	
Pigs (9/18)	778 ^b	147 to 2288	23.1 ^b	1.9 to 236	34	5
Laying hens (8/18)	754 ^b	137 to 11169	62 ^b	2.5 to 12282	12	
Turkeys (3/6)	2100 ^b	502 to 4408	362 ^b	94 to 762	6	
Sawmills ^c (10/10)	402.5 ^b	229.7 to 604.3	271.1 ^b	166.8 to 671.6	1.5	Present study

a - geometric mean; *b* - median; *c* - endotoxin analysed in total dust; *d* - endotoxin analysed in inhalable dust; *e* - endotoxin analysed in respirable dust

m⁻³) proposed by The National Health Council of the Netherlands (DECOS) (9) and above the occupational exposure limit of 12.5 ng m⁻³ (125 EU m⁻³) set by the International Commission on Occupational Health (ICOH) (10). As Rylander and Carvalho have proposed 10 ng m⁻³ (100 EU m⁻³) as the threshold for airway inflammation (11), endotoxin levels in our study may be hazardous for the respiratory system.

A wide range of endotoxin levels in airborne dust in woodworking industry has been described in literature, mainly for inhalable and total dust.

They vary with occupational settings and climate, including the type of woodworking process (e.g. wet vs dry wood processing), dust levels, and atmospheric conditions (temperature, air humidity and flow). The influence of sampling methods, transport conditions, and methods of extraction and analysis must also be taken into account (12). Most of these studies found endotoxin levels to exceed exposure limits (13-17) and were similar to our results for endotoxin in airborne dust in similar sampling conditions (wet wood processing over the summer). Low endotoxin levels

(<50 EU m⁻³) were found only in Swiss (18) and Swedish (19) sawmills. All of these studies assessed exposure only to airborne dust endotoxin. Our study has ventured further with measurements of settled dust endotoxin in order to see the relation between airborne and settled dust endotoxin levels. Similar comparisons have been made before in various settings, but not in woodworking industry (Table 2). They suggest that settled dust can be an indoor reservoir of endotoxins, and therefore a health hazard, should it be raised by indoor activities (20, 21). Our study showed a similar endotoxin load in airborne and settled dust but no significant correlation between the two. The ratio between median endotoxin levels in settled and airborne (respirable) dust was only 1.5. Similar low ratios were found in poultry houses for turkeys and laying hens (6 and 12, respectively), where settled dust is expected to be highly disturbed (5). In contrast, this ratio in households, aircraft cabins, and cattle stables is usually between 80 and 125, suggesting only minor disturbance of floor endotoxin reservoirs (5, 21, 22).

To conclude, this study has pointed to sawmill settled dust as a relevant endotoxin reservoir and has suggested that it may add to already high exposure to airborne endotoxins associated with wood processing. Our initial results of endotoxin exposure in Croatian sawmills suggest that the relation between settled and airborne dust endotoxin levels need to be further investigated to better understand the sources and sites of endotoxin contamination in sawmills, and the risk posed by endotoxin reservoirs in settled dust to human health in particular. This knowledge could change the way we assess endotoxin exposure and could have practical implications on safety at work and health protection in wood-processing industry and other environments.

Acknowledgement

This study was supported by the grant number 022-0222411-2410 financed by the Ministry of Science, Education and Sports of the Republic of Croatia.

REFERENCES

- Rylander R. Endotoxin in the environment-exposure and effects. *J Endotoxin Res* 2002;8:241-52.
- Liebers V, Raulf-Heimsoth M, Brüning T. Health effects due to endotoxin inhalation. *Arch Toxicol* 2008;82:203-10.
- Douwes J, Thorne P, Pearce N, Heederik D. Bioaerosol health effects and exposure assessment: progress and prospects. *Ann Occup Hyg* 2003;47:187-200.
- Hyvärinen A, Roponen M, Tiittanen P, Laitinen S, Nevalainen A, Pekkanen J. Dust sampling methods for endotoxin - an essential, but underestimated issue. *Indoor Air* 2006;16:20-7.
- Schierl R, Heise A, Egger U, Schneider F, Eichelser R, Nesper S, Nowak D. Endotoxin concentration in modern animal houses in southern Bavaria. *Ann Agric Environ Med* 2007;14:129-36.
- Workplace atmospheres – Guidance for sampling in inhalable, thoracic and respirable aerosol fractions (CEN/TR 15230:2005)
- US Food and Drug Administration (FDA). Guideline on validation of the *Limulus* amoebocyte lysate test as an end-product endotoxin test for human and animal parenteral drugs, biological products and medical devices 1987 [displayed 29 March 2010]. Available at <http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM080966.pdf>.
- Varnai VM, Macan J, Plavec D, Jureša D. Endotoxin measurement in house dust using the end-point *Limulus* amoebocyte lysate method. *Arh Hig Rada Toksikol* 2004;55:175-81.
- Dutch Expert Committee on Occupational Standards (DECOS). Health-based recommended occupational exposure limit for endotoxins. Publication no. 1998/03WGD. Rijswijk: Health Council of the Netherlands; 1998.
- Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J, Donham K, Palmgren U, Nowak D. Air contaminants in different European farming environments. *Ann Agric Environ Med* 2002;9:41-8.
- Rylander R, Carvalheiro MF. Airways inflammation among workers in poultry houses. *Int Arch Occup Environ Health* 2006;79:487-90.
- Spaan S, Heederik DJ, Thorne PS, Wouters IM. Optimization of airborne endotoxin exposure assessment: effects of filter type, transport conditions, extraction solutions, and storage of samples and extracts. *Appl Environ Microbiol* 2007;73:6134-43.
- Mandryk J, Alwis KU, Hocking AD. Effects of personal exposures on pulmonary function and work-related symptoms among sawmill workers. *Ann Occup Hyg* 2000;44:281-9.
- Rongo LM, Msamanga GI, Burstyn I, Barten F, Dolmans WM, Heederik D. Exposure to wood dust and endotoxin in small-scale wood industries in Tanzania. *J Expo Anal Environ Epidemiol* 2004;14:544-50.
- Duchaine C, Thorne PS, Mériaux A, Grimard Y, Whitten P, Cormier Y. Comparison of endotoxin exposure assessment by bioaerosol impinger and filter-sampling methods. *Appl Environ Microbiol* 2001;67:2775-80.
- Douwes J, McLean D, van der Maarl E, Heederik D, Pearce N. Worker exposures to airborne dust, endotoxin and beta(1,3)-glucan in two New Zealand sawmills. *Am J Ind Med* 2000;38:426-30.
- Dennekamp M, Demers P, Bartlett K, Davies H, Teschke K. Endotoxin exposure among softwood lumber mill workers in the Canadian province of British Columbia. *Ann Agric Environ Med* 1999;6:141-6.
- Oppliger A, Rusca S, Charrière N, Vu Duc T, Droz PO. Assessment of bioaerosols and inhalable dust exposure in Swiss sawmills. *Ann Occup Hyg* 2005;49:385-91.

19. Dahlqvist M, Johard U, Alexandersson R, Bergström B, Ekholm U, Eklund A, Milosevich B, Tornling G, Ulfvarson U. Lung function and precipitating antibodies in low exposed wood trimmers in Sweden. *Am J Ind Med* 1992;21:549-59.
20. Bouillard L, Michel O, Dramaix M, Devleeschouwer M. Bacterial contamination of indoor air, surfaces, and settled dust, and related dust endotoxin concentrations in healthy office buildings. *Ann Agric Environ Med* 2005;12:187-92.
21. Hines CJ, Waters MA, Larsson L, Petersen MR, Saraf A, Milton DK. Characterization of endotoxin and 3-hydroxy fatty acid levels in air and settled dust from commercial aircraft cabins. *Indoor Air* 2003;13:166-73.
22. Park JH, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environ Health Perspect* 2001;109:859-64.

Sažetak**IZLOŽENOST ENDOTOKSINU U DRVOPRERAĐIVAČKOJ INDUSTRIJI: ODNOS IZMEĐU RAZINA IZMJERENIH U ZRAKU I NA TLU**

Drvoprerađivačka industrija radna je sredina s izloženošću velikim količinama bioaerosola bogatog endotoksinom, što je povezano s učincima na zdravlje izložene radne populacije. Cilj ove studije bio je procijeniti odnos između razina endotoksina u sedimentiranoj prašini i zraku (aerosoliziranoj prašini) drvoprerađivačkih radnih prostora. Deset parova uzoraka zraka i sedimentirane prašine skupljeno je u dvije tvornice za preradu drva (pilanama i pogonima za proizvodnju parketa) u Hrvatskoj. Endotoksin je izmjeren kromogenom "end-point" LAL (*Limulus* amebocitni lizat) metodom. Rezultati su pokazali da razine endotoksina u aerosoliziranoj respirabilnoj prašini drvoprerađivačkih pogona prelaze preporučene granice izloženosti za radne prostore od 125 EU m⁻³, pa se mogu smatrati štetnima za dišni sustav izloženih radnika. Razine endotoksina izmjerene u sedimentiranoj i aerosoliziranoj prašini kretale su se u sličnu rasponu [(229.7 do 604.3) EU mg⁻¹ i (166.8 do 671.6) EU m⁻³], ali nije utvrđena značajna korelacija razina endotoksina između parova uzoraka sedimentirane i aerosolizirane prašine (Spearman rho=0.358, P=0.310). Ovi rezultati upućuju na to da uz velike količine proizvedene i aerosolizirane drvene prašine u sklopu radnih operacija u pilanama, razini endotoksina u aerosoliziranoj respirabilnoj prašini može pridonijeti i uskovitlana sedimentirana prašina koja je dodatni rezervoar endotoksina. Istraživanje odnosa između razina endotoksina u sedimentiranoj i aerosoliziranoj prašini treba nastaviti kako bi se bolje definirali izvori i mjesta kontaminirana endotoksinom u pilanama.

KLJUČNE RIJEČI: *aerosolizirana prašina, bioaerosoli, LAL-test, proizvodnja parketa, pilana, sedimentirana prašina*

CORRESPONDING AUTHOR:

Jelena Macan, MD, PhD
Occupational Health and Environmental Medicine Unit
Institute for Medical Research and Occupational Health
Ksaverska cesta 2, 10000 Zagreb, Croatia
E-mail: jmacan@imi.hr