Endotoxins and glucans

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Outline

- Definition & associated health effects
- Determination
- Reported levels in office buildings

Endotoxins

- Fragments of cell walls of all Gram negative bacteria
- Complex lipo-polysaccharides

  Released after lysis of micro-organism

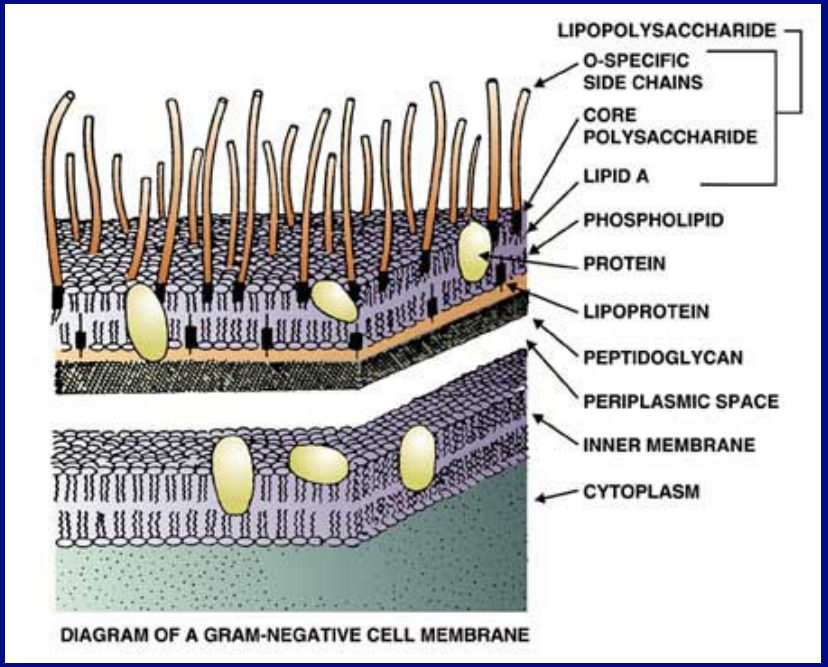
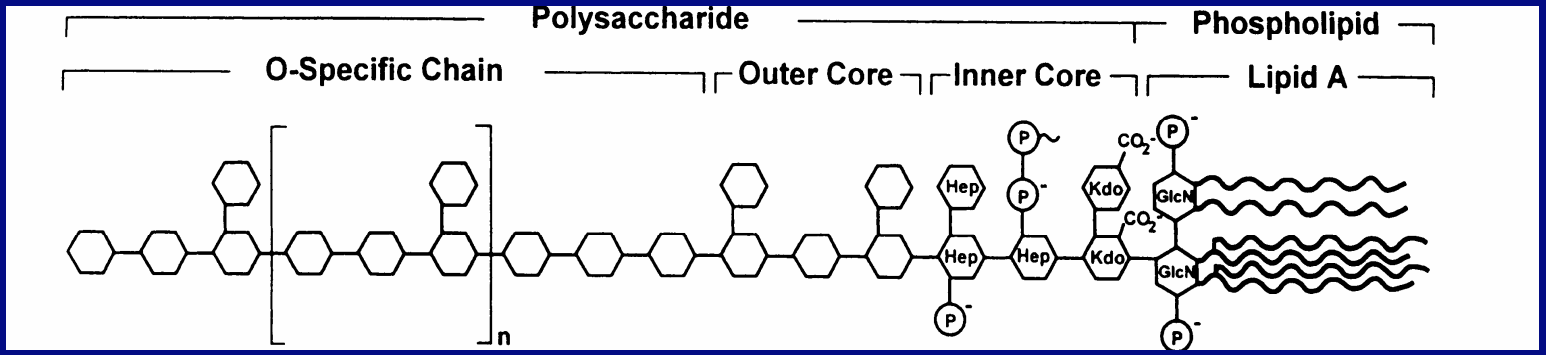


DIAGRAM OF A GRAM-NEGATIVE CELL MEMBRANE

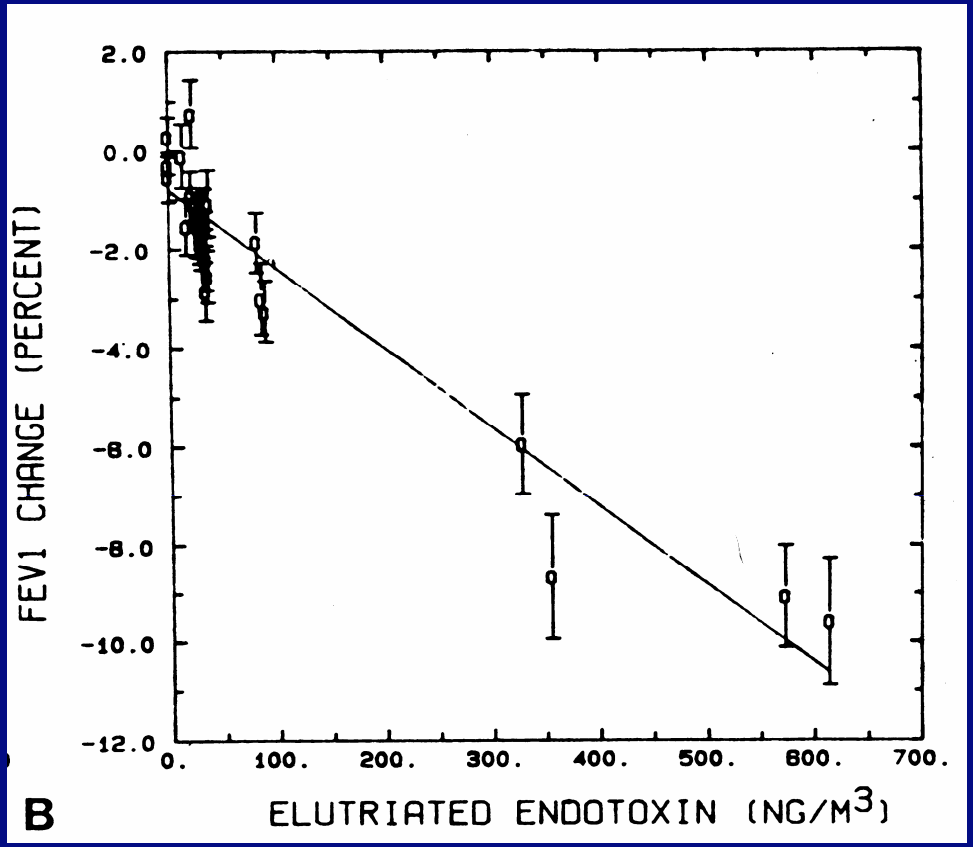


Health effects of LPS

- "*Endotoxins are read by our tissues as the very worst of bad news*" and "*in response to these molecules we are likely to turn on every defence at our disposal*" (Thomas 1974)
- Non-allergenic cell wall component with strong pro-inflammatory properties
- Occupational endotoxin exposure has been associated with ***adverse respiratory and systemic effects*** in children and adults
- Very early environmental endotoxin exposure has been suggested to ***protect against atopy and asthma***

Occupational Exposure Limits for endotoxin

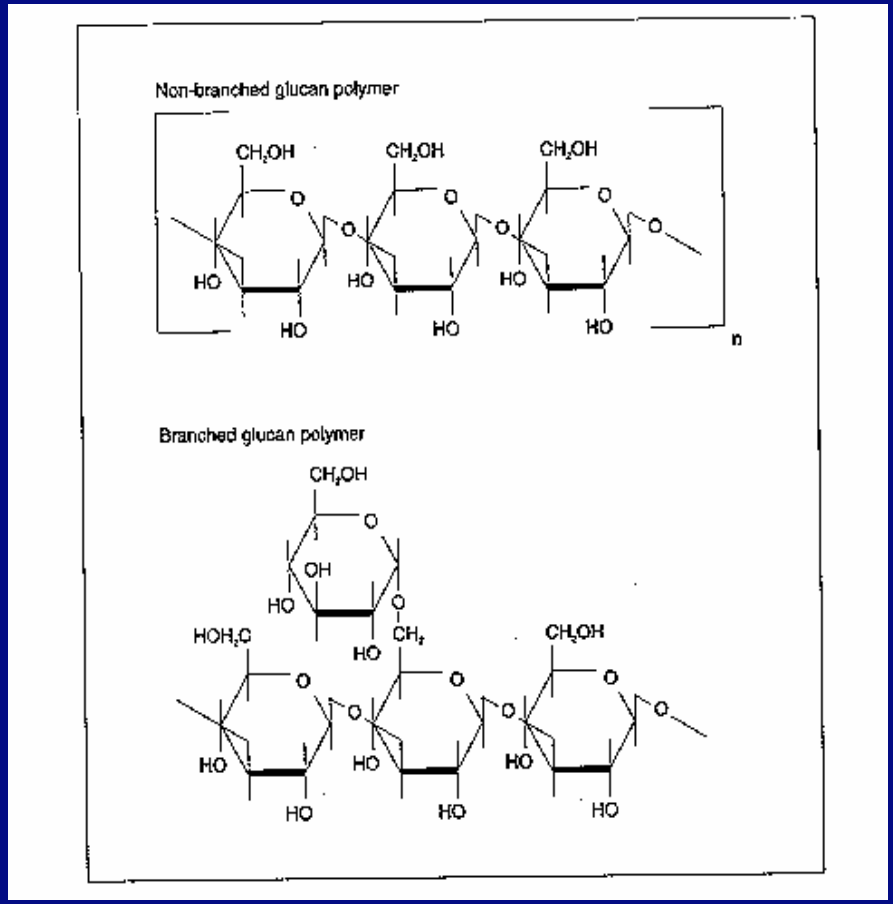
- Observational studies on acute respiratory effects suggest LOELs/NOELs below 100 EU/m^3
  (Milton 1994, 1995; Zock 1998)
- Dutch recommended health based OEL is set at 50 EU/m^3 based on a challenge study with cotton dust (LOEL ≈ 90 EU/ m^3)
  (DECOS 1998)
- Dutch legal limit is set at 200 EU/m^3; effective from Januari 2003 withdrawn faesibillity issues



(Castellan 1987)

ß(1,3)-glucans

- Polyglucose molecules [ß(1⌧3)-linked] with highly variable structures
- Important cell wall components of most molds, some bacteria and many plants
- fungi and yeasts: ß(1⌧3)(1⌧6)-glucans
  plants: ß(1⌧3)(1⌧4)-glucans
  water insoluble
- Commonly present in many occupational environments but also in the general environment



Health effects of ß(1,3)-glucans I

- Inducers of inflammatory reactions
  neutrophils, macrophages and complement (in vitro),
  cytokines (whole blood)
  enhancement of host-mediated induced resistance to infections and antitumor activity (in vivo)

- In native form non-immunogenic

  ß(1⌫3)-glucan-specific receptor on human macrophages

Endotoxin & Glucan exposure assessment in office building

- Collection:
  Inhalable & Surface dust (floors & chairs)
- Extraction
- Assay

Measurement method (NEN-EN 14031)

- Classical inhalable dust sampling
- Extraction filter with dust (rocking/ shaking 1 hour at RT centrifugation 15 min at 1000 x G)
- kinetic chromogenic or turbidemtric version of Limulus Amebocyte Lysate (LAL) assay
- Results in Endotoxin Units/m^3

GC/MS assay not widespread

Handling of samples not uniquely defined by NEN-protocol

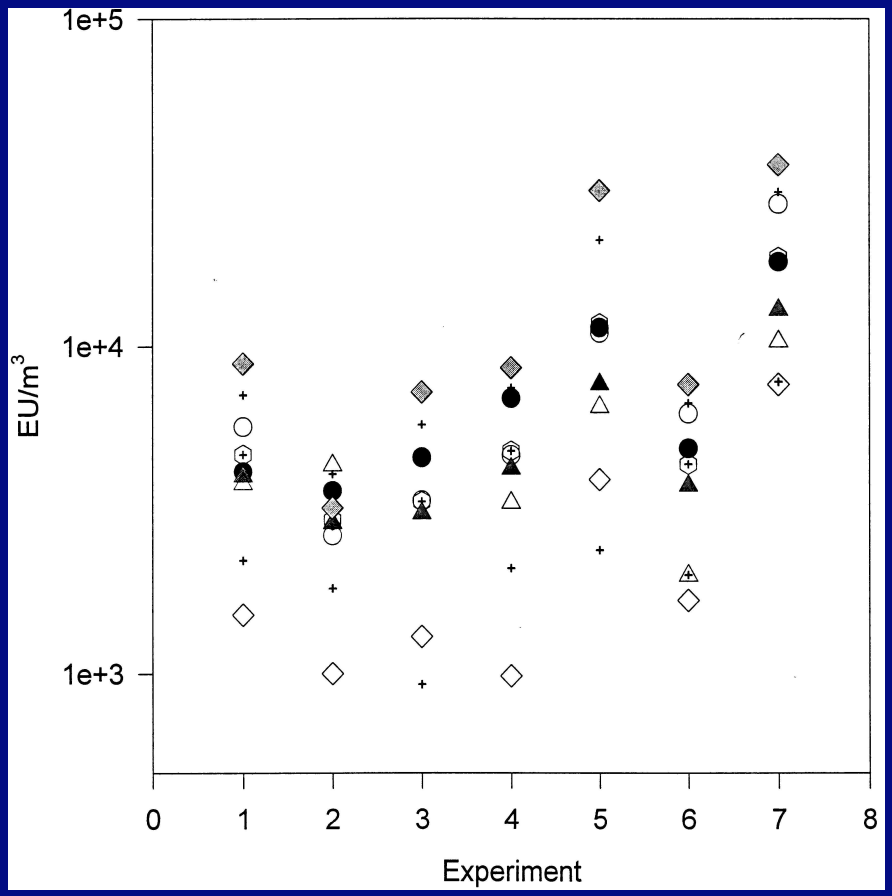
- Transportation: under dry conditions, preferably with dehumidifier; >24 hr dehumidified or frozen.
- Storage: when not extracted within a few days, store at –20 (avoid additional growth of microbes; keep cool and dry)
- Extraction fluids: maximize yield (with or without detergents)
- Storage of extracts : at 4 C (analysis next day) or at –20C (prolonged storage)

Comparability of laboratory results

Total protocol
(assay type,extraction)
(Reynolds et al., 2002; Chun et al., 2002)

- overall high correlation
- systematic differences dependent on type of dust (< factor 10)

Performance improves when protocols are harmonized
(Chun et al., 2002 and Linsel et al., 2003)



(Reynolds et al., 2002)

Endotoxin analysis – The influence of filter and extraction media (Douwes et al., 1995)

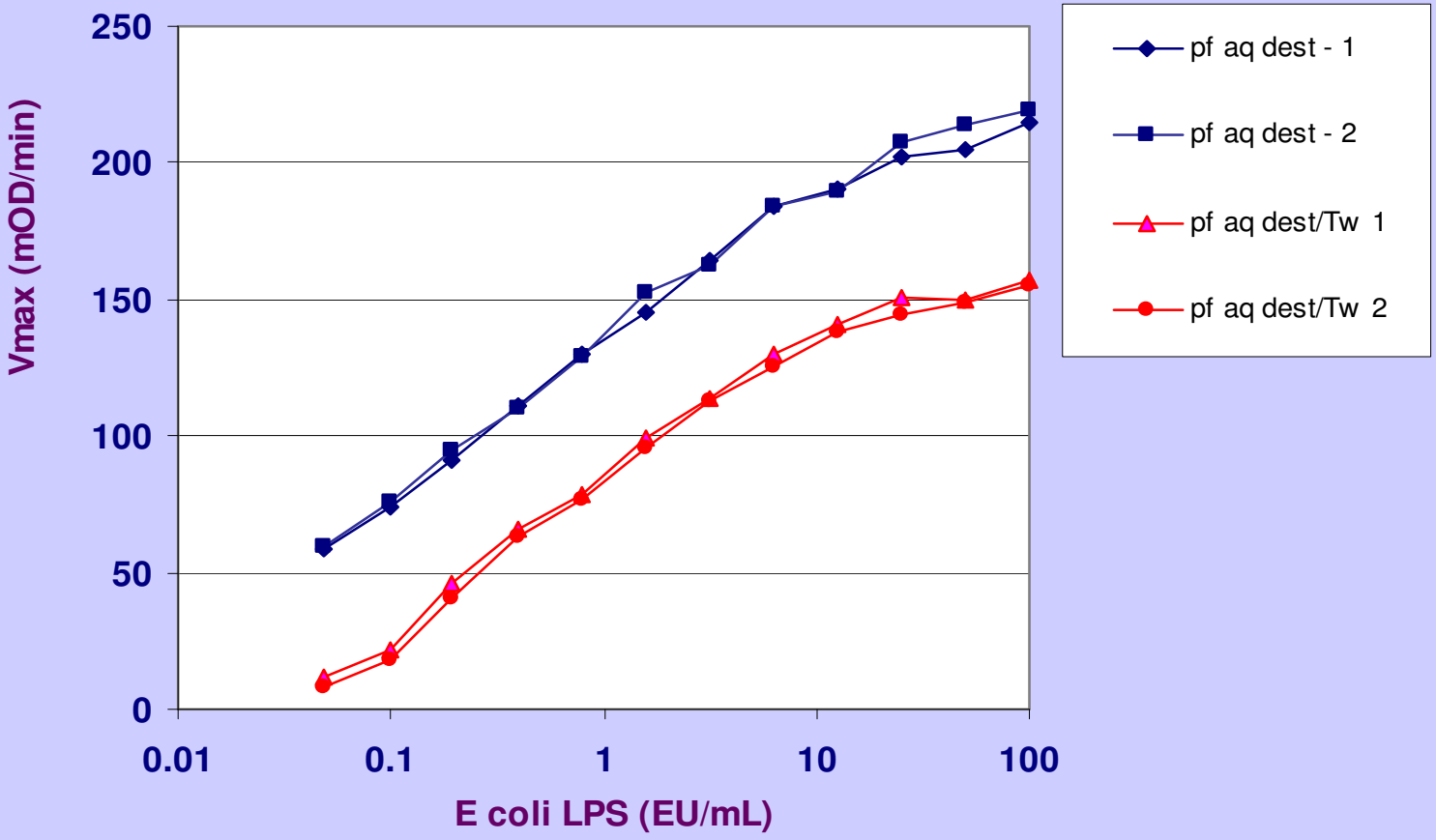
		ratio	95% CI
Extraction medium	0.05% Tween 20	**7.2**	6.3-8.3
	Pyrogen-free water	**1.0**	
Filter type	Glass fibre	**2.3**	1.9-2.8
	Teflon	**2.0**	1.7-2.5
	Polycarbonate	**1.8**	1.5-2.2
	Cellulose ester	**1.0**	

Based only on dust from potato-processing industry

Tween induces a shift in standard curve

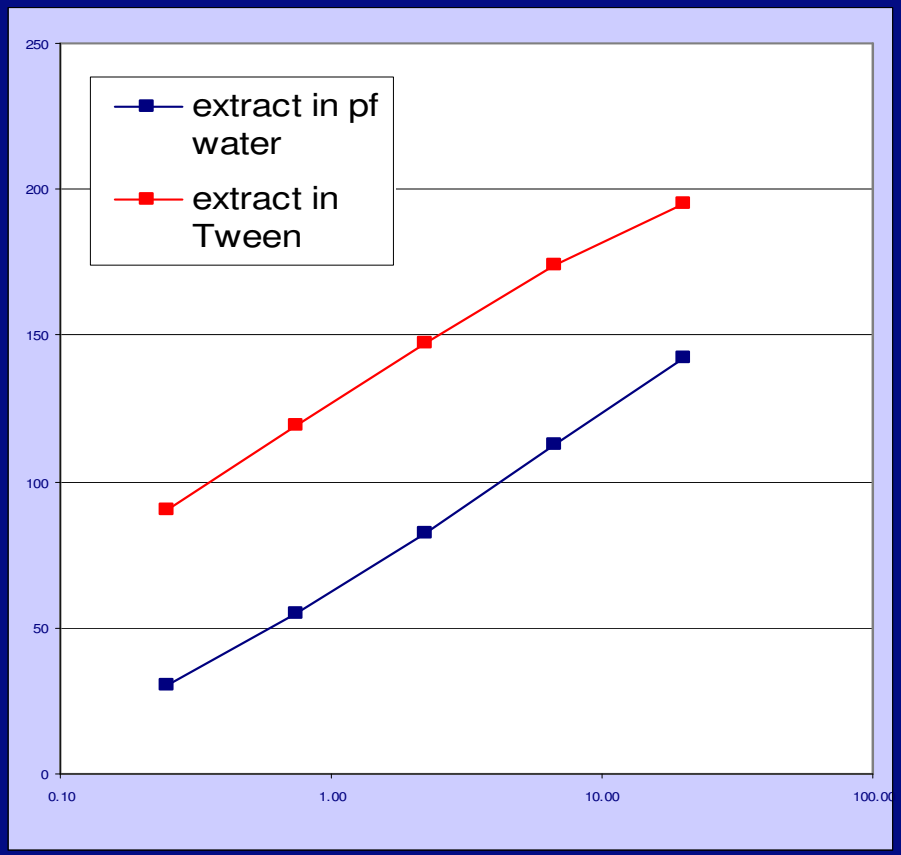
Fig. 1

Reactivity of standard LPS (E.coli) in LAL assay: effect of Tween-20 (0.05%) in assay medium

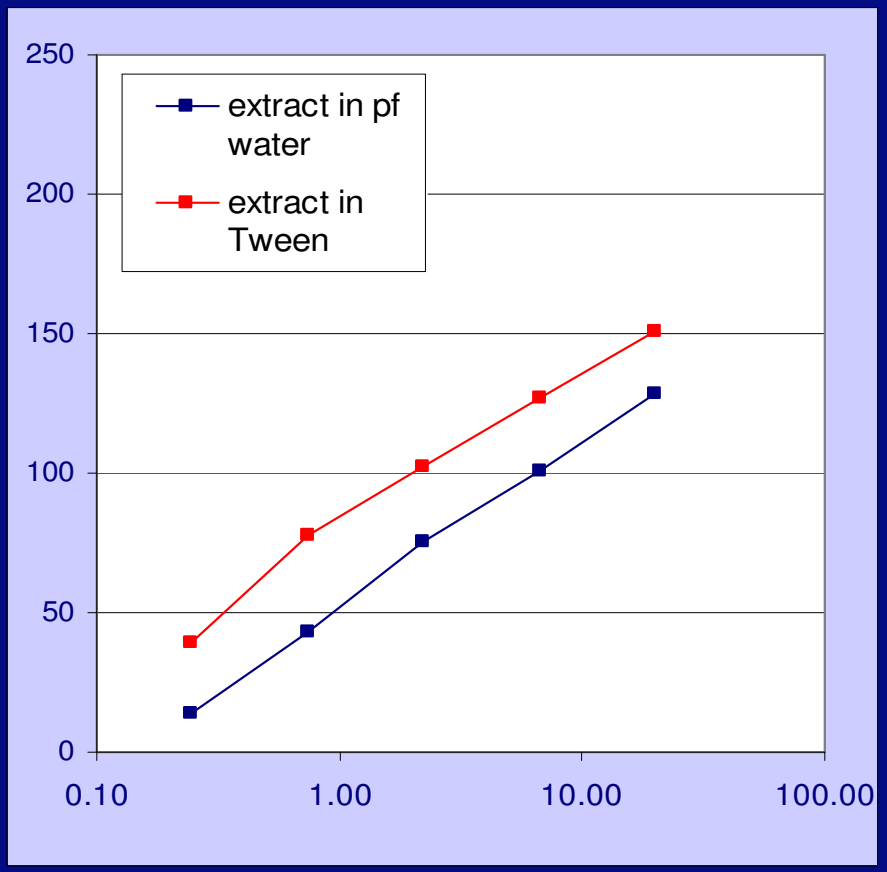


School (*) dust LPS reactivity (Vmax) in LAL assay: *effect Tween-20 in extraction and/or assay medium*

LAL assay in water



LAL assay in water-Tween



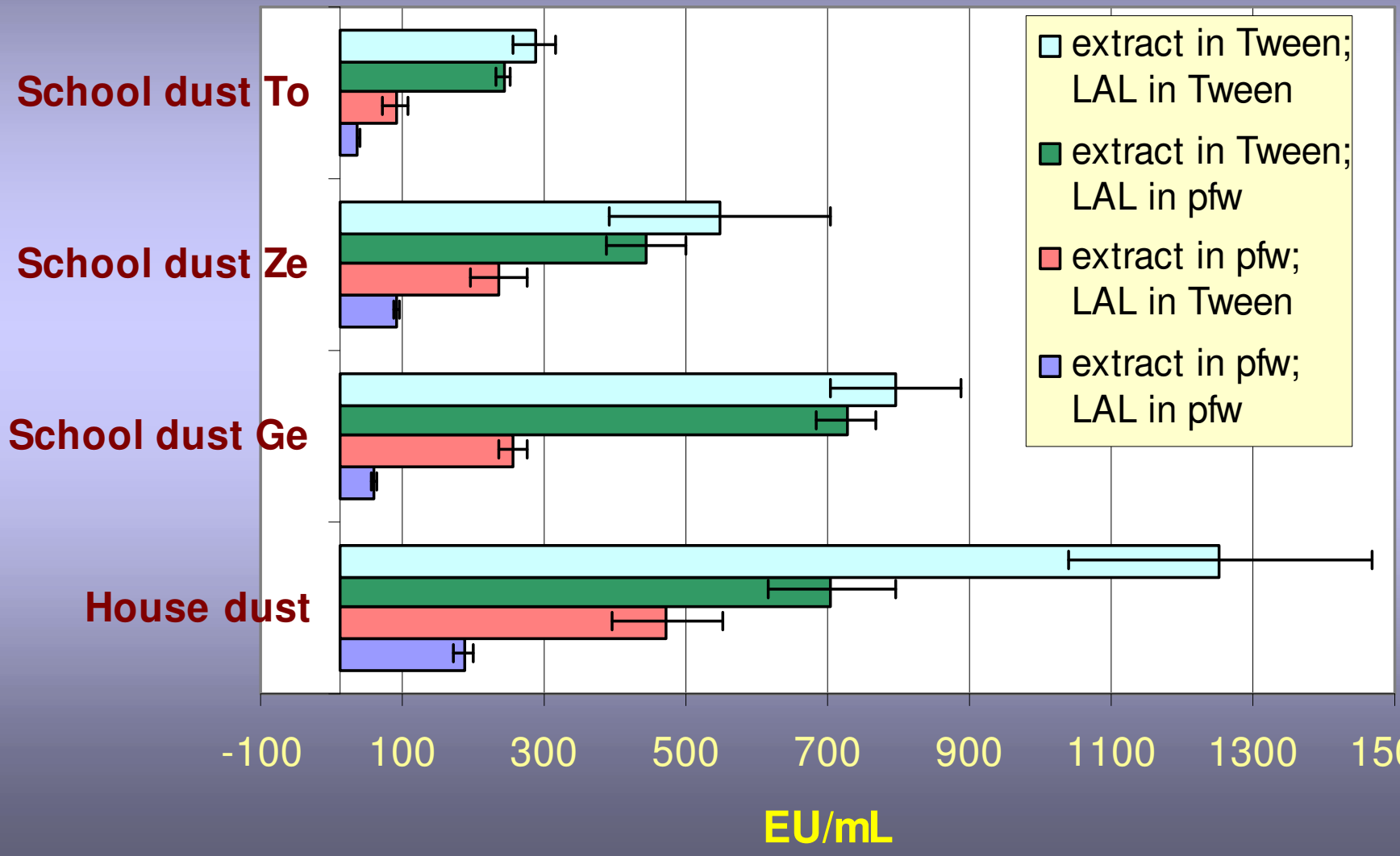
dust extract (microliter/mL) in LAL

(*) school GE

Independent of extraction, shift when Tween in assay

Measured LPS content in environmental dust:

effect Tween in extraction and/or LAL assay



Other recommendations, based on practical issues

- Transportation: under dry conditions, preferably with dehumidifier; >24 hr dehumidified or frozen.
- Storage: when not extracted within a few days, store at –20 (avoid additional growth of microbes; keep cool and dry)
- Extraction fluids: maximize yield (with or without detergents) Extraction Tween, Assay without Tween
- Storage of extracts : at 4 C (analysis next day) or at –20C (prolonged storage)

ß(1,3)-glucan analysis

Glucan-specific LAL test
(Aketagawa et al, 1993, and others)

- Extraction 0.3 M NaOH
- Commercially available
- Expensive
- Sensitive but not entirely specific

Glucan-specific inhibition enzyme immuno assay (Douwes et al, 1996)
Sandwhich immuno assay (Milton et al. 2001)

- Extraction 0.3 M NaOH or heat
- not commercially available
- Very specific but not very sensitive
- Comparability to LAL not known

Glucan studies (Douwes, Indoor air 2005)

Reference/environment	Exposure measurements: *n*	Assay	Concentration (range or means)	Studied health effects: No. subjects	Associated with exposure	Not associated with exposure
Indoor environment						
Rylander et al., 1992	46	LAL	Problem buildings: 0.2–0.55 ng/m^3	39	Dry cough ↑; skin rashes ↑[a]	Nose and eye irritations; che
Schools, post office, day care	36		Control building: <0.1 ng/m^3	405		tightness; head ache; tiredn joint pains, etc.
Rylander, 1997a[†]	24	LAL	Before renovation: 11.4 ng/m^3	11	Airway hyperreactivity ↑[a]	Lung function; symptoms
Day care center	13		After renovation: 1.2 ng/m^3			
Thorn and Rylander, 1998a	75	LAL	0–19 ng/m^3	129	Atopy ↑[b]; serum MPO ↑; FEV_1↓[b]	Atopy[c]; Airway hyperreactivit
Row houses						ECP; C-reactive protein; FEV symptoms
Rylander et al., 1998[†]	6	LAL	Problem school: 15.3 ng/m^3	65	Cough ↑; cough with phlegm ↑,	Atopy
Schools	11		Control school: 2.9 ng/m^3	141	hoarseness ↑	
Wan and Li, 1999	?	LAL	Day care centers: 5.7 ng/m^3	40	Lethargy/fatigue ↑	Eye and nose irritations, skin
Day care, Offices, homes			Office buildings: 3.2 ng/m^3	69		and respiratory symptoms
			Homes: 3.7 ng/m^3	22		
Douwes et al., 2000a	69	ELISA	Non-symptomatic children: 126 ng/m^2	69	PEF variability in symptomatic	No other health effects
Homes	74		Symptomatic children: 169 ng/m^2	74	children ↑[d]	were studied
Beijer et al., 2003*	17	LAL	High exposed: 6 ng/m^3	17 high exp	Cytotoxic CD8+ T-cells ↑;	BMC secretion of IL-10 and
Row houses					IFN-γ/IL-4 ratio after in vitro	Il-1β, serum ECP, MPO, IFN-
	18		Low exposed: 0.9 ng/m^3	18 low exp	stimulation of BMNCs ↑[b]	and IL-4; differential cell counts in blood; symptoms

Reported levels in NL office buildings

- Glucans not reported in the Netherlands and abroad
- Endotoxin only 1 study in NL, more abroad

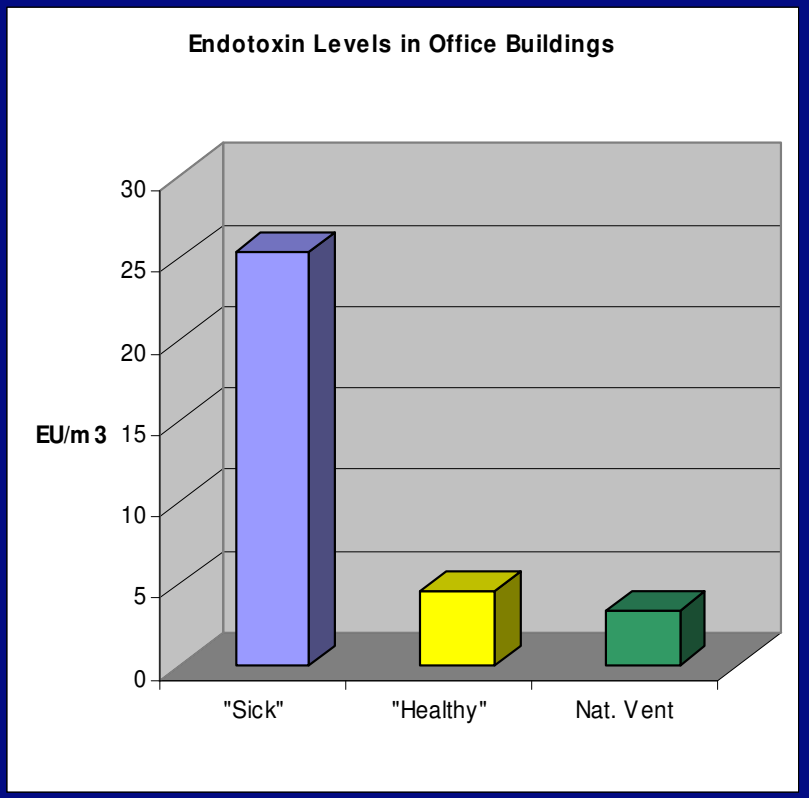
Dutch office buildings

1335 workers in 19 buildings

Sick: symptom prevalence>=15%

Healthy: symptom prevalence < 15%

- Six times higher compared to healthy buildings
- Seven times higher compared to natural ventilated buildings
- Teeuw et al. 1994



Endotoxin in non-problem US office buildings (Reynolds et al. 2001)

TABLE XIII

Kruskal-Wallis chi-square approximations for number of symptoms and environmental parameters (n = 6)

Parameters	Men		Women	
	χ^2	p	χ^2	p
Carbon dioxide	0.92	0.34	1.77	0.18
Temperature	**4.68**	**0.03**	0.37	0.54
Relative humidity	1.27	0.26	**3.46**	**0.06**
Carbon monoxide	**3.20**	**0.07**	0.00	0.94
Noise	**4.05**	**0.04**	1.29	0.26
VOCs	0.39	0.54	0.05	0.82
Formaldehyde	**3.40**	**0.06**	0.12	0.73
Acetaldehyde	**3.44**	**0.06**	0.02	0.90
Fungi	1.46	0.22	0.23	0.63
Mesophilic bacteria	0.04	0.83	0.06	0.80
Thermophilic bacteria	1.46	0.23	0.23	0.63
Endotoxin	**3.13**	**0.08**	**2.93**	**0.09**
PM10	**3.69**	**0.05**	0.04	0.84

TABLE VII

—PM10, endotoxin, and total bioaerosols[A]

	Geometric mean (GSD)		
	PM10 ($\mu g/m^3$)	Endotoxin (EU/m^3)	Total bioaerosols ($organisms/m^3$)
	25 (1.0)	0.9 (1.4)	Not quantifiable[B]
	<0.1 (0)	0.5 (1.4)	10,700 (10.8)
	36 (1.0)	0.7 (2.7)	8,850 (2.6)
	20 (1.0)	1.3 (1.3)	509 (3.5)
	16 (1.0)	3.0 (1.9)	1,520 (1.5)
	14 (1.0)	1.4 (1.1)	1,370 (1.0)
) =	0.04	<0.01	0.27

cell for all samples. Duplicates at one location were
veraging over multiple locations.
umerate.

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doi:10.1111/j.1600-0668.2005.00415.x

Fungal and endotoxin measurements in dust associated with respiratory symptoms in a water-damaged office building

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National Institute for Occupational Safety and Health

Table 2 Arithmetic means and ranges of floor-specific geometric mean levels of culturable fungi and endotoxin in floor dust within each exposure category

	Tertiles of exposure		
Unit of measurements	Low ($n = 5$)	Medium ($n = 5$)	High ($n = 5$)
Average levels (ranges) of culturable fungi			
cfu/mg floor dust	4.9 (3.9–5.8)	7.9 (6.2–8.9)	12.5 (10.0–21.6)
cfu/m^2 floor area	800 (600–1200)	1800 (1400–2100)	4400 (2200–7800)
Average levels (ranges) of endotoxin			
EU/mg floor dust	3.5 (2.3–4.6)	8.1 (5.7–10.5)	33.7 (12.7–65.6)
EU/m^2 floor area	700 (200–1200)	2400 (1800–3300)	6700 (4100–10,400)

N=888 questionnaire

N=338 exposure; median floor levels ranked

Synergistic effects endotoxin and fungi

Table 4 Interaction effect of exposure to culturable fungi and endotoxin in floor dust on work-related lower and upper respiratory symptoms

	No interaction models[a]		Interaction models[b]		
			Exposure to[c]		
Symptoms	Exposure[c]	OR (95% CI)	Fungi	Endotoxin	OR (95% CI)
Wheeze			High	Low	1.2 (0.43–3.35)
	Fungi	1.8 (1.02–3.00)	Low	High	1.9 (0.68–5.33)
	Endotoxin	2.8 (1.62–4.81)	High	High	3.8 (1.59–9.16)
Chest tightness*			High	Low	1.1 (0.46–2.69)
	Fungi	1.8 (1.12–3.04)	Low	High	1.3 (0.52–3.25)
	Endotoxin	2.2 (1.37–3.63)	High	High	3.0 (1.42–6.32)
Attacks of shortness of breath**			High	Low	0.7 (0.27–1.77)
	Fungi	2.0 (1.14–3.51)	Low	High	0.7 (0.25–1.93)
	Endotoxin	2.3 (1.35–3.85)	High	High	2.4 (1.13–5.07)
Shortness of breath when hurrying**			High	Low	0.6 (0.21–1.75)
	Fungi	1.6 (0.89–2.93)	Low	High	0.9 (0.32–2.73)
	Endotoxin	2.5 (1.40–4.57)	High	High	2.3 (0.99–5.24)
Cough with phlegm			High	Low	1.2 (0.46–3.16)
	Fungi	1.4 (0.82–2.30)	Low	High	1.9 (0.73–5.00)
	Endotoxin	2.2 (1.30–3.65)	High	High	2.7 (1.20–6.27)
Stuffy, itchy, runny nose, sneezing			High	Low	1.3 (0.66–2.38)
	Fungi	1.3 (0.90–1.96)	Low	High	1.9 (0.96–3.75)
	Endotoxin	2.0 (1.40–2.92)	High	High	2.6 (1.50–4.53)
Throat irritation			High	Low	1.5 (0.77–3.01)
	Fungi	1.4 (0.93–2.09)	Low	High	1.6 (0.79–3.34)
	Endotoxin	1.5 (1.00–2.15)	High	High	2.2 (1.20–3.90)

In summary

Glucan and endotoxin is measured in dust

Health based reference values for endotoxin

However, associations between symptoms and levels found in office buildings or homes at much lower levels

Is endotoxin the causative factor or surrogate marker???