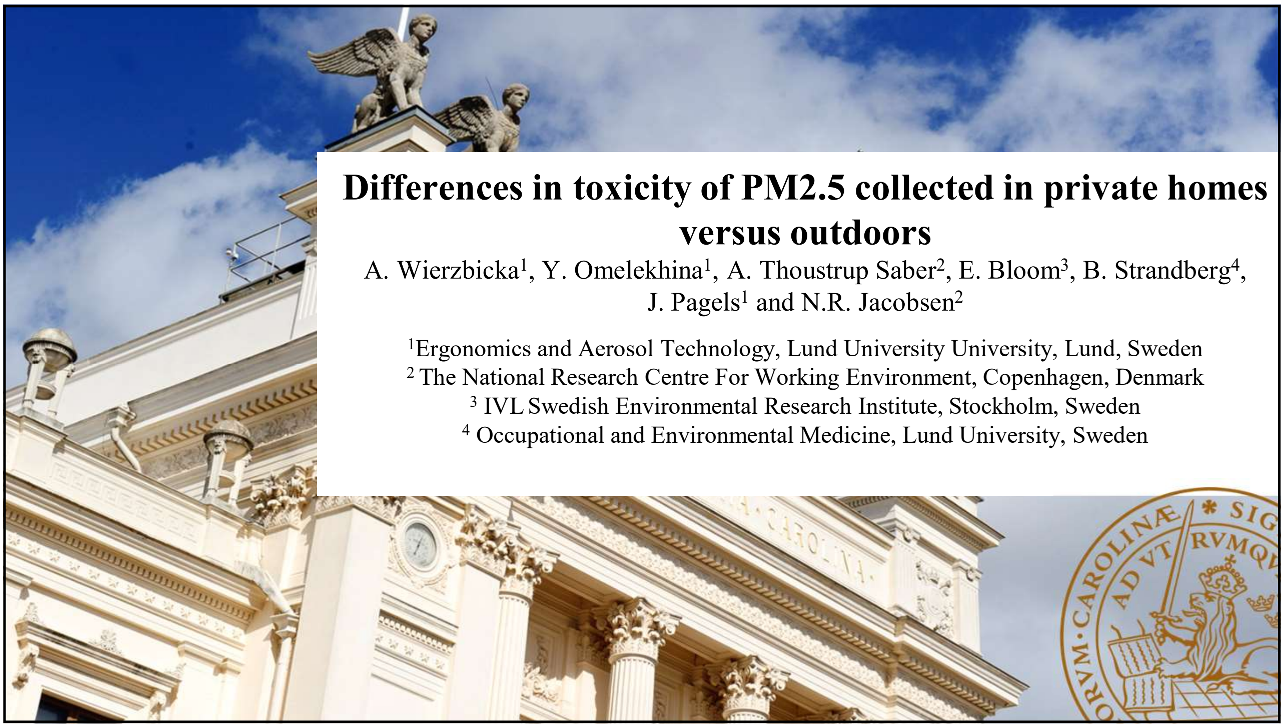

Differences in toxicity of PM2.5 collected in private homes versus outdoors

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Facts / puzzle

- Epidemiological studies – strong correlation between particle mass concentration and health effects
- Tox studies – strong evidence for UFP toxicity
- In indoor environments powerful indoor sources contribute to high UFP number concentrations….
- We spend majority of the time indoors

In epidemiological studies assessing health effects of exposure to airborne particles, indoor environments are considered as places where people are exposed to particles of outdoor origin.

Should it remain this way?

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Do the indoor sources matter at all from health effects perspective?

Toxicity of indoor particles?

- Particles collected indoors had higher cytotoxic effects on mouse macrophages than particles collected outside one single family house in Finland (Happo et. al., 2013, 2014)
- Long et al, 2001 – proinflamatory response (bioassays - rat alveolar macrophages) higher for indoor particles than outdoor particles (14 paired samples in Boston area)
- Oeder et al., 2012 indoor PM10 from school compared with outdoor PM10 induced more inflammatory and allergic reactions, and accelerated blood coagulations
- Skovmand et al., 2017 candle light particles caused higher inflammation and cytotoxicity in the mice lungs (after intratracheal instillation) than diesel exhaust particles

Aims

1. To determine differences in toxicity of particles inside and outside occupied residences by conducting toxicological studies in mice
2. To assess physico-chemical properties of airborne particles inside and outside occupied residences in Sweden

Wierzbicka et al, 2022, Indoor Air, 32:e13177

Measuremnets

- Measurements in 15 occupied residences
- Week long measurements between October 2016 – April 2017
- Measuremnets were conducted simultanously inside and outside
- Instructions were given to occupants to ensure that periods with active indoor sources were captured
- Occupants were asked to keep log books
- Air exchange rates were assessed and building characteristics gathered

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Measurements

NanoTracer
particle number concentration, mean size (10-300 nm)

DustTrack DRX
Proxy mass conc., PM2.5 particles collection for gravimetric, PAHs, metals and ions analysis

Dekati Gravimetric Impactor, PM2.5
for toxicological studies

Mini-aethalometer
black carbon mass concentration

PM2.5 collection
for endotoxins analysis

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PM2.5 particles collected for tox studies

(70 mm filters, 70 l/min flow, Dekati Gravimetric Impactor)

- Gravimetric analysis
- Extraction (twice: 30 ml methanol, ultrasonic water bath, 30 min, below 35 deg C)*
- Pooling (separately: inside, outdoors, blanks)
- Vacuum drying (35deg C and 150 mbar)*
- Extracted and dried particles used in tox mice studies
- Analysis of pooled samples: PAHs, metals, soluble ions, endotoxin, OC/EC

* Ruusunen et al, 2011, Anal Bioanal Chem 401:3183–3195

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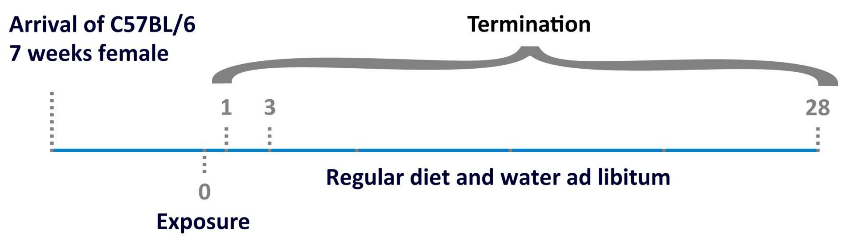
Toxicological testing in mice

Female C57BL (N=162 mice, in each group 6 mice per dose)

Mice received a single intratracheal instillation of 18, 54 and 162 μg of the pooled particle samples. Suspended in NanoPure water with 0.1% Tween80

Carbon Black Printex 90/XE-2B was used as a positive control



Lung inflammation, genotoxicity, and acute-phase response in lung were evaluated 1, 3 and 28 days after intratracheal instillation.

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Home No.	Apartment/ house	Floor area (m^2)	Volume (m^3)	Type of ventilation	AER (h^{-1})	Number of residents	Occupancy (%)
1	House	285	645	Natural[a]	0.56[c]	3	84
2	Apartment	85	212	FTX	1.18[c]	2	79
3	House	250	625	Natural[a]	0.39[c]	7	76
4	House	110	275	Natural[a]	0.57[c]	4	91
5	Apartment	117	322	Natural[a]	0.52[c]	4	99
6	Apartment	66	164	Mechanical[b]	0.50[d]	1	–
7	Apartment	66	164	Mechanical[b]	0.59[d]	2	84
8	Apartment	86	215	Mechanical[b]	0.31[d]	2	85
9	Apartment	66	164	Mechanical[b]	1.60[c]	3	77
10	Apartment	66	164	Mechanical[b]	0.40[d]	1	94
11	Apartment	86	215	Mechanical[b]	0.41[d]	1	–
12	Apartment	87	218	Mechanical[b]	0.31[d]	4	–
13	Apartment	46	115	Mechanical[b]	0.51[d]	1	64
14	Apartment	80	200	FTX	0.85[c]	4	73
15	Apartment	46	115	Mechanical[b]	0.64[d]	3	94

Abbreviations: AER, air exchange rate; FTX, exhaust and supply air ventilation with heat recovery.

[a]Equipped with kitchen hood.

[b]Mechanical exhaust ventilation system.

[c]Measured by tracer gas method.

[d]Exhaust airflows measurements.

Wierzbicka et al, 2022

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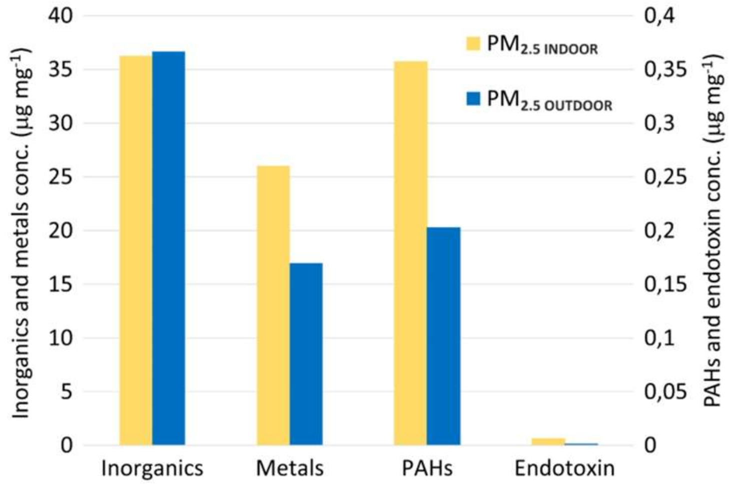
Chemical composition of extracted PM2.5 particles



Inorganics (Si, P, Na, K, and Ca), metals (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, tot-As, Cd, Ba, Tl, Pb, and Mg), PAHs (16 priority US EPA PAHs) and endotoxins

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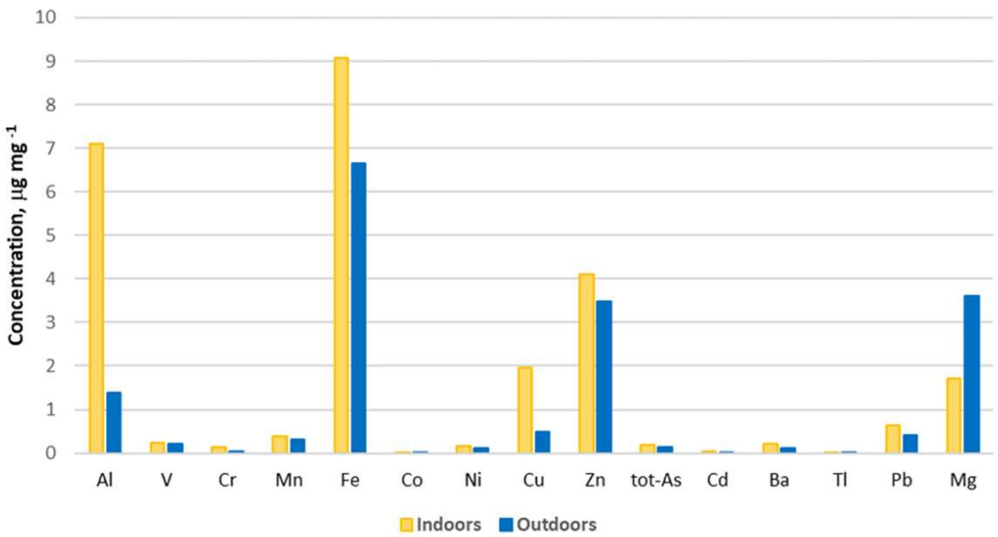
Metals (µg/mg)



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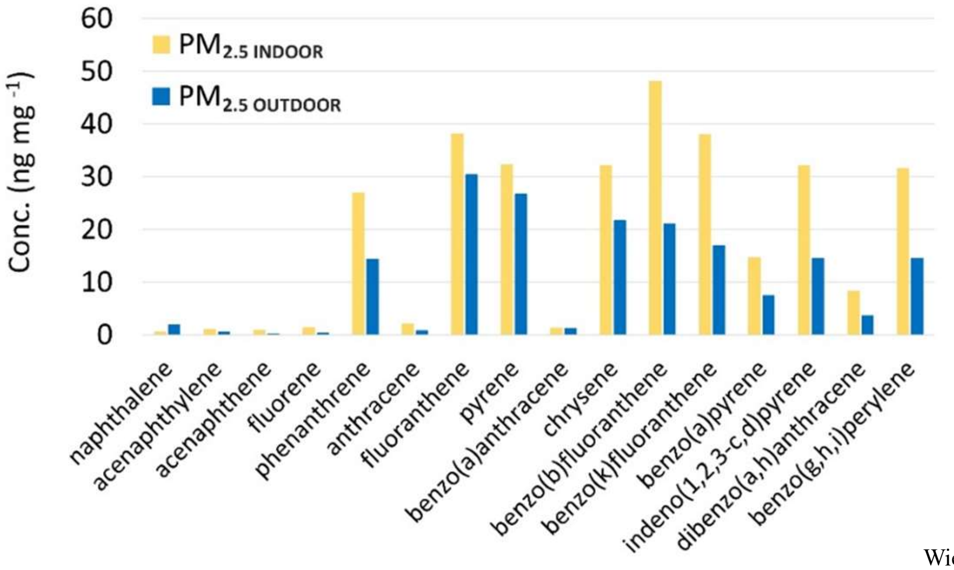
PAHs 16 priority U.S. EPA



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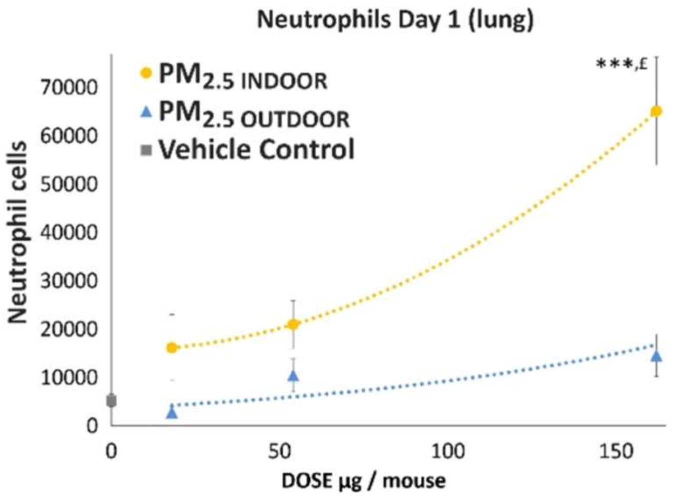
Endotoxins

	Indoors		Outdoors	
	ng/m^3	ng/mg	ng/m^3	ng/mg
Individual filters				
Average	0.29	42.6	0.08	11.4
Range (min - max)	0.06 - 0.69	1.7 - 118.9	0.03 - 0.19	0.8 - 23.1
Ratio I/O	3.6	3.7		
Extracted from filters, used for tox studies				
Pooled filters		6.3		1.3
Ratio I/O		4.8		

Suggested limit value in occupational env: 90 EU/m^3 (ca 9 ng/m^3), 2011 Arbetsmiljöverket,
The Nordic Expert Group

Observed effects

In broncheoalveolar lavage (BAL) after a single intratracheal instillation of 18, 54 and 162 μg of particles



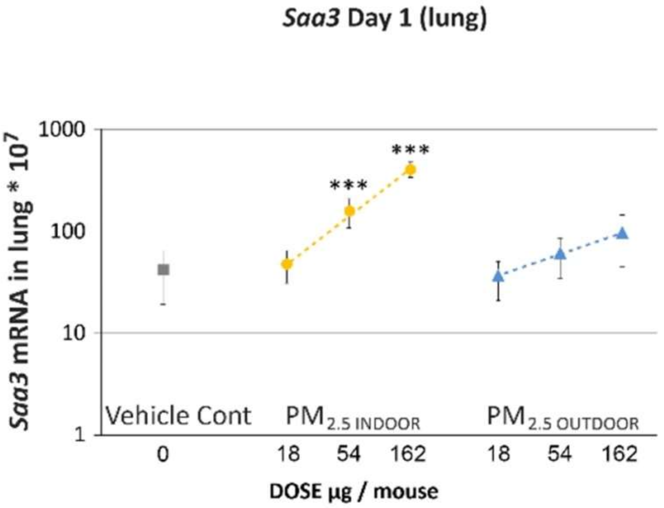
*** Statistically significant increase compared to control mice at the 0.001 level.
£ Statistically significant increase compared to $PM_{2.5\ OUTDOOR}$ exposed mice at the 0.05 level

Printex 90 at 162 μg dose:
After 1 day: 98490 Neutrophils cells

Wierzbicka et al, 2022

Observed effects

mRNA expression levels of Saa3 in the lung tissue



*** Statistically significant increase compared to control mice at the 0.001 level.

Printex 90 at 162 μg dose:
After 1 day: $10000*10^7$

Wierzbicka et al, 2022

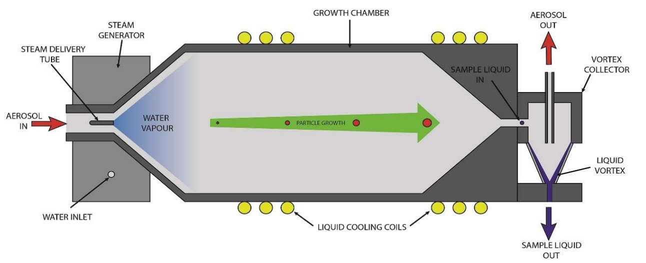
Conclusions

- Higher concentrations of metals, PAHs and endotoxins were observed indoors than outdoors
- $PM_{2.5}$ indoors caused significantly higher lung inflammation and lung acute- phase response 1 day post-exposure compared to $PM_{2.5}$ outdoors
- None of the tested materials caused genotoxicity.
- $PM_{2.5}$ indoors displayed higher relative toxicity than PM2.5 outdoors under the studied conditions: wintertime with reduced air exchange rates, high influence of indoor sources, and relatively low outdoor concentrations
- Reducing exposure indoors requires reduction of both infiltration from outdoors and indoor-generated particles

Wierzbicka et al, 2022

Future outlook

- More studies on toxicity of particles are needed
- Is assessment Reactive Oxygen Species (ROS) – a way forward? Pre-screening of toxicity and/or possible alternative health relevant PM metric?
- We have built, in cooperation with scientists from Quensland Univerity of Technology (Australia), an instrument to measure ROS in real time and assess ROS off-line. We invite all to cooperation



Brown et al ., 2019, Atmos. Meas. Tech, 12, 2387-2401

- Based on 9,10-bis (phenylethynyl) anthracene-nitroxide (BPEAnit) ROS assay,
- 1 min resolution,
- sensitivity to a broad range of ROS

Acknowledgment

This work was financed by The Swedish Research Council Formas

Particle number concentrations (10-300 nm) in cm^{-3} and I/O ratio

№	Total total monitoring period					Occupancy time			Non-activity time		
	Indoors		Outdoors		I/O ratio	Indoors	Outdoors	I/O ratio	Indoors	Outdoors	I/O ratio
	Average	SD	Average	SD		Average			Average		
1	11700	42100	2600	500	4.5	12300	1300	9.5	1800	1200	1.5
2	7400	19300	3800	1400	1.9	8600	4000	2.2	3200	3300	1.0
3	4700	9700	4300	2300	1.1	5300	4000	1.3	2000	4300	0.5
4	10900	32400	4400	2600	2.5	11200	4400	2.5	1900	3500	0.5
5	4300	4500	5300	4100	0.8	4300	5300	0.8	2200	3800	0.6
6	1000	500	1800	1400	0.6	-			-		
7	8600	27300	3100	1400	2.8	9600	3000	3.2	2000	3100	0.6
8	6300	15700	2600	1300	2.4	6800	2500	2.7	1800	2700	0.7
9	38900	120830	1900	900	20.5	49500	1900	26.1	1600	1800	0.9
10	13400	75800	1300	600	10.3	14200	1300	10.9	1000	1100	0.9
11	-					-			-		
12	6400	41000	1500	1200	4.3	8200	1600	5.1	1300	1400	0.9
13	5400	19600	1400	800	3.9	6200	1400	4.4	1200	1400	0.9
14	2700	3800	3100	7500	0.9	2900	2700	1.1	2100	3900	0.5
15	2500	14000	3000	15900	0.8	2600	3100	0.8	900	1700	0.5
Average	**8900**		**2900**		**4.1**	**10900**	**2800**	**5.4**	**1800**	**2500**	**0.8**
SD	**9300**		**1400**			**11900**	**1300**		**600**	**1200**	

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PM2.5 mass concentrations in µg m-3 and I/O ratio

№	Total monitoring period					Occupancy time			Non-activity time		
	Indoors		Outdoors		I/O ratio	Indoors	Outdoors	I/O ratio	Indoors	Outdoors	I/O ratio
	Average	SD	Average	SD		Average			Average		
1	3.5	10.6	5.0	1.1	0.7	-			-		
2	3.2	2.2	5.3	3.3	0.6	3.2	5.6	0.6	1.0	4.6	0.2
3	3.7	4.5	5.3	6.2	0.7	4.5	4.9	0.9	2.0	6.5	0.3
4	5.2	6.2	4.4	5.0	1.2	5.2	4.4	1.2	1.7	4.1	0.4
5	6.0	13.4	6.8	6.0	0.9	6.0	6.8	0.9	2.2	6.8	0.4
6	2.3	2.3	4.0	3.6	0.6	-			-		
7	11.7	12.1	15.1	9.0	0.8	11.7	15.1	0.8	12.3	17.3	0.7
8	15.6	12.7	21.9	15.6	0.7	14.3	21.2	0.7	12.6	22.4	0.6
9	10.8	28.2	10.2	10.0	1.1	11.0	10.2	1.1	10.0	10.2	0.6
10	5.5	20.8	6.5	3.9	0.8	5.6	6.5	0.9	1.6	5.9	0.3
11	7.5	-	4.7	-	1.6	-			-		
12	5.2	3.6	5.5	4.7	0.9	7.3	5.9	1.2	5.2	5.3	1.0
13	24.3	189.2	3.7	3.7	6.6	24.3	6.3	3.9	2.7	6.5	0.4
14	2.1	1.2	5.0	3.7	0.4	2.3	5	0.5	2.0	5.0	0.4
15	5.5	5.2	6.6	20.2	0.8	5.7	6.6	0.9	1.5	4.8	0.3
Average	**7.5**		**7.3**		**1.2**	**8.4**	**8.2**	**1.1**	**4.6**	**8.3**	**0.5**
SD	**6.0**		**5.0**			**6.2**	**5.0**		**4.4**	**5.7**	

Wierzbicka et al, 2022